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INHERITANCE IN WHEAT.

I. AN "UNFIXABLE WHEAT" (INVESTIGATIONS
ON THE LATE M. PHILIPPE DE VILMORIN'S
"RACE DE BLÉ NAIN INFIXABLE").

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(With One Plate.)

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§ 1. VILMORIN'S MATERIAL AND RESULTS.

WHILE making selections from commercial stocks of wheat in 1902, Vilmorin found, in two cases, a distinctive dwarf plant. Besseler's Brown Clubhead was the first stock, and the second a wheat derived from a cross made in 1886 but regarded as "parfaitement fixée." Approving the sturdy straw and general characteristics of these dwarfs he propagated them, but in each case failed to secure fixity of type. From the seeds of the original dwarf plant there grew tall plants which on selfing bred true, and dwarf plants which on selfing "split" into tall and dwarf. These phenomena were repeated from generation to generation. Observations on these "ever-splitting" forms were continued on rather non-analytical lines for some ten years, and upon them Vilmorin⁽¹⁾ expressed the following general opinions:

(i) The original dwarf plants probably resulted from natural cross pollination. For this he had no evidence.

(ii) Tall and dwarf could not be infallibly discriminated by measurement. This was apparent from the progenies.

(iii) Tall plants, when selfed, bred true. In the progenies of selfed dwarfs were tall and dwarfs.

(iv) The tall : dwarf ratio in the progenies of selfed dwarfs, fluctuated between 1 : 2.5 and 1 : 4.0. But consideration should be given to the possibility of a 1 : 2 : 1 ratio distorted by type-fluctuation and by low viability of the third type in the ratio.

(v) In the progenies of selfed dwarfs were, invariably, a certain number of bushy plants which never came to ear. They might be survivors of the low-viability type postulated in the 1 : 2 : 1 ratio explanation.

(vi) Neither germination tests nor counts of post-germination survival afforded help in explaining the phenomenon.

In 1920 material originally derived from Vilmorin's second dwarf—the one which had appeared suddenly in a wheat of hybrid origin—was received from the John Innes Horticultural Institution, Merton¹. This furnished the cultures whose behaviour on selfing is described in the passages which follow.

In the first year of investigation it was apparent that the progenies of dwarf plants showed segregation not only for height of plant but also for ear density. In general the lax-eared plants were distinctly taller than the dense. Among wheats generally, ear density is less fluctuating and therefore more amenable to accurate observation than height of plant. Consequently from the outset it was made the prime experimental variable.

Preliminary investigation showed the need for a three-fold inquiry. First, a strict diagnosis of genetic types was required: then inter-type ratios would need to be settled: finally, pollen and grain viability would have to be examined, and general corroboration sought by suitable hybridisations.

To test the constancy of inter-type ratios comprehensive numerical data had to be collected and submitted to tests of probability. Numerical

¹ [Observations on this strain had been made by Mr M. A. Bailey, assisted by several members of the staff at Merton, for some years. This experience agreed with and extended that of M. de Vilmorin in various respects. Since, however, Mr Bailey, at the conclusion of the war, took up an appointment in Egypt, and was unable to continue these experiments, Mr Engledow began an independent study of the surviving material.—W. B.]

matter so enhances cost of publication that it has been considered justifiable to omit from this account the actual plant- and culture-data. This, fortunately, was of a nature to facilitate and justify the general statement of results and probabilities.

§ II. THE GENETIC TYPES.

Without dwelling on the elaborate detail of investigation which the determination of genetic types involved, it is essential to describe these types. A wide range of material was studied, and in every generation single plant progenies were kept separate. The facts will be best appreciated by considering the progeny of what came to be called a normal dense-eared plant. Such a progeny always embraced four types:

- (i) Lax-eared plants.
- (ii) Dense-eared plants.
- (iii) Stunted plants with a single elongated stem from which, however, no ear emerged.
- (iv) Bushy plants, profusely tillered, with narrow, dark green leaves. These never formed elongated stems under ordinary conditions, and almost invariably died out some weeks before plants of the other types were ripe to harvest.

Type (i), lax, bred true to laxness throughout the experiment. As between lax cultures derived from single lax or dense plants of the original Merton stock, no constant differences were observable.

Type (ii), dense, on self-fertilisation, invariably gave all four types (i)-(iv).

From types (iii) and (iv) seed was unobtainable under ordinary conditions. By growing under special conditions, however, more was subsequently learned of these types.

Certain special features of the four types must now be considered. In any population of lax plants, whether from a lax or dense parent plant, ear density and height of plant showed considerable fluctuation. This appeared not markedly greater than is commonly observed in wheat forms generally. But as type-discrimination was of the essence of the "ever-splitting" problem, all fluctuations were closely studied.

Repeated observation over four years led to the conclusion that all plants classed by eye as lax were of a single, true-breeding, genotype. It is noteworthy that the average height of the lax plants was about one and a third times that of the type (ii) dense plants. But actual measurements of plant stature disclosed an overlap of the type (i) and type (ii) distributions so marked as to discourage attempts to confirm ear-density classification by observation of height.

Dense-eared plants [type (ii) above] were more difficult material. When normally developed they could be classed as "dense" without hesitation. But every year, in all dense-containing cultures, the dense plants exhibited very marked fluctuation in height and general development. This fluctuation was not only more marked than in the case of lax plants, but exceeded that shown by the generality of wheat forms under similar conditions. In four successive years numerous selections of dense plants were made, taking many gradations of height and general development. From these it was clear that the small impoverished plants, yielded progenies strictly comparable with those from the largest and most vigorous. Finally it appeared beyond doubt that all plants classed by eye as dense were of one genotype.

Yet greater difficulties were met in type (iii), the stunted plants with one elongated stem, resembling a flowering stem, from which no ear emerged. In many cases it was possible to dissect out an imperfect ear. All such ears, though distorted, were recognisably dense. In addition, the foliage and habit of these stunted plants bore a good general resemblance to the normal dense plants of type (ii). It was therefore finally concluded that the type (iii) plants were simply poor specimens of dense plants. Thus types (ii) and (iii) are to be regarded as a single genotype characterised by an extreme fluctuability in general development amounting in some cases to premature death.

There remained type (iv), the bushy, sterile, pigmies. These were studied in field plots, in pot-growings, and from winter and spring sowings. Since some insect and fungoid parasites, for example, the "wheat rosette" described by McKinney⁽²⁾, induce a bushy growth, the action of such agencies was carefully sought. No clue resulting from any of these inquiries, only one possibility appeared to remain. The pigmy plants might represent a third genotype having an inordinately long developmental period. Such a one could not come into ear if sown in the usual months of October or November. Moreover, being tardy in growth, it necessarily suffered from the taller lax and dense plants growing in the same culture. Accordingly in 1923, seeds from some ordinary dense

plants were sown on August 18th. As soon as certain type-discrimination was possible—in mid-June—all plants other than the pigmies were uprooted and removed. Elongated stems were slowly developed on the remaining bushy pigmies and, some three weeks after the time for ear-emergence in the lax and dense types, ears appeared from them. They were very distinct, being shorter and much denser than the familiar type (ii) dense, and resembled *Triticum compactum* (the club wheats). The flowering stems of these pigmies were very sturdy but of less than half the height found in type (ii) dense plants. Unfortunately no grains were formed, the plants being apparently self-sterile. To whatever cause this first recorded flowering of the bushy pigmies should actually be attributed, it seems quite certain that the plants represent a third and distinct genotype. To regard them as homozygous for ear density, the type (ii) dense being heterozygous and the type (i) lax, as is known, homozygous, is a strong temptation. But this possibility must be left over until type ratios are under discussion.

Pl. I, with the notes, will give a good general idea of the ear shapes which have been mentioned.

There are believed to be then, in the progeny of selfed dense plants, three separate genotypes—lax, dense, and pigmy-dense. Of these, dense and pigmy-dense fluctuate very widely, a feature which must be remembered as one of their salient genotypic characteristics.

§ III. THE "EVER-SPLITTING" RATIO.

While the characteristics of the type occurring in the progenies of dense plants were being examined, observations were also made on the frequency ratios of these types. The progenies of single plants were kept separate and the statistical probability of every frequency was carefully checked.

1921 *Crop*. In the drought of 1921 all the pigmy plants [type (iv), § II] were dead before harvest as also were many of the stunted type (iii). Consequently counting was confined to plants with one or more visible ears. Classification was first into lax and dense. Then the dense plants were divided into two sub-classes based upon height. But the ratio of frequencies for these two sub-classes in individual cultures was extremely fluctuant. Trial showed, in fact, that only the ratio (lax):(total plants having an ear) possessed any suggestive constancy. For the combined progenies of all dense plants the average value of this ratio was 48.6 per cent. This, for simplicity, was regarded as representing 50 per cent.

actual value. Altogether 588 plants representing twelve groups of single plant cultures were observed, each group having come from an original single plant. The deviation from 50 per cent. of the lax:total plant ratio in all the twelve culture groups in turn was less than $1.5 \times$ standard error of sampling. Compared with the expectation of chance, the distribution of deviations was:

Extent of Deviation	Found	Expected
0.0—1.0 S. error	8 cases	8.19 cases
> 1.0 S. error	4 cases	3.81 cases
	<u>12</u>	<u>12</u>

So far, then, 50 per cent. was an acceptable value. Further, the average found for the whole population (48.6 per cent.) deviates from 50 per cent. by only 1.4 per cent., which is less than the standard error of sampling (2.06 per cent.). But an equally good fit would probably have been obtained to 49 per cent. or 51 per cent., so that the special recommendation of 50 per cent. is simplicity.

It is to be remembered that all earless plants [types (iii) and (iv) of § II] were excluded from this first year's count.

1922 Crop. With the combined objects of diagnosing genotypes and testing ratio constancies, more extensive growings were made in 1922. To meet the difficulty of some plants dying out before harvest, a first count was made on the growing material shortly after ear emergence was complete. A second confirmatory count was made upon the harvested material. For the green count, six arbitrary classes of plant were recognised:

1 and 3 = lax ear of two grades of height.

2 and 4 = dense ear of two grades of height.

5 = bushy pigmy plants.

6 = stunted plants with an elongated stem from which no ear was protruded.

To decide whether all the classes represented distinct genotypes, their frequency ratios were carefully studied. The results, now to be discussed, suggested certain combinations among the six classes and a three-fold classification was finally adopted. This, of course, was independently supported by the result already recorded in § II.

In all, 3361 plants belonging to 43 cultures, were arranged in the six arbitrary classes mentioned above. Then for every culture in turn certain frequency-ratios were determined, *e.g.* frequency of class 1: frequency of class 3. For the 43 culture-values of any one ratio the mean (M), the standard-deviation (σ) and the coefficient of variation

($100\sigma/M$) were calculated. This coefficient, being a critical index to ratio constancy, afforded strong presumptive evidence as to the genetic identity or distinctness of the six arbitrary plant classes. A further test of constancy was applied. Every inter-class ratio in turn was calculated for the complete population of 43 cultures, and the deviation of every separate culture value from this population average value was determined. Then the actual distribution of deviations was compared with that expected on the chance basis. This use of the population average value for each ratio was, of course, quite arbitrary. But neither the number nor relationship of the genuine genetic types being known, no other practicable procedure could be devised.

Only the outstanding features of the results will be given. Frequencies in the arbitrary sub-classes for the complete population of 3361 plants were:

Classes 1 and 3 = 46.9 per cent. and 2.4 per cent.
 2 and 4 = 36.5 per cent. and 3.2 per cent.
 5 = 4.2 per cent.
 6 = 6.8 per cent.

It will be observed that class 5 (pigmyies) and class 6 (stunted ear-less plants) were small proportions of the population. Moreover, they were both inconstant proportions. For both of them, the distribution of culture deviations from the population average value, was of a kind not attributable to chance. And further, the coefficients of variation for culture-values of the proportions were nearly 70 per cent.

Three ratios were satisfactory in respect of separate culture deviations from the population average. These were, denoting the frequency of class x by (x) :

$$\frac{(1)}{(1)+(2)} \quad \frac{(1)+(3)}{(1)+(2)+(3)+(4)} \quad \frac{(1)+(3)}{\text{Total plants}}$$

and the coefficients of variation evaluated in the manner already described:

17.39

18.09

16.84

Consideration of all the ratios created a strong presumption in favour of $(1) + (3) : \text{total plants}$, *i.e.* the proportion of all plants with lax ears to the total population. This implied a recognition of only two types—lax and the rest. Meanwhile study of diagnostic characters in selected single plant cultures had also suggested that all lax-eared plants were of a single genotype, the arbitrary height sub-division having only a fortuitous basis. But it had suggested that in plants other than lax were at least two types. The bushy pigmyies were certainly different from the

other non-lax plants. If they represented a separate genotype, the great inconstancy of their proportion in separate cultures would be presumptive evidence of their low and fluctuating viability.

For the combined 43 cultures the ratio lax : total plants was 49.3 per cent., and 48.97 per cent. for the average of the separate culture values. Thus it was close to the 48.6 per cent. obtained in the 1921 count, from which, however, classes 5 and 6 of the 1922 count were excluded.

At harvest all plants were carefully gathered. Since the green count a few stunted plants had protruded ears, in all cases dense. This fact helped to justify the union of classes 2, 4, and 6, described above, which had been decided on from other observations. Allowing for the stunted plants which had subsequently produced ears, the classification of the ripe material strongly corroborated the results of the earlier count. The value of lax : total plants was 49.4 per cent. as against 49.3 per cent. in the earlier count.

1923 Crop. Observations were continued on the lines adopted in 1922, but only the harvested material was observed. Substantially the same conclusions were reached as to genotypic identity and ratio constancies. But the ratio lax : total plants was 54.1 per cent. as against the 49 per cent. of previous years. Remembering the characteristic mortality among the pigmy and the stunted plants, some explanation of the disparity as a seasonal effect might be attempted. Certainly the seasons of 1921-2-3 were very different, but no critical evidence of their influence can be adduced. It is therefore best simply to accept the 1923 crop value as evidence that the ratio is about 50 per cent., but has a considerable seasonal fluctuation. To explore the uncertainty further would be theoretically desirable, but past experience showed the impracticability of substantial refinement in the methods so far followed.

1924 Crop. Only 535 plants were observed as in this year a subsidiary hybridisation test engaged attention (see § IV). The ratio lax : total was 46.1 per cent. Here again is evidence of year to year fluctuation which may spring from direct seasonal influence on plant growth, or, alternatively, may reflect some more subtle cytological effect.

The investigations described in this and the preceding paragraphs thus led, by an inevitably circuitous route, to the following defensible conclusions:

Dense-eared plants, on self-fertilisation, yield a progeny containing

three genotypes—lax, dense, and pigmy. Lax-eared plants continue to breed true. Dense-eared plants are characteristically fluctuant in general development, in some cases remaining stunted and failing to protrude ears. On selfing they yield three types again. Pigmies are bushy plants, and under ordinary conditions fail even to produce elongated, apparent flowering stems. Under special conditions, in 1924 for the first time, they were grown to the ear-bearing stage. The ear was denser than that of the normal dense-eared plants. The ratio of genotypes in the progeny of normal dense plants is lax : dense : pigmy \approx 50 per cent. : 46 per cent. : 4 per cent. but it is liable to considerable year to year fluctuation. Height of plant characterises the three genotypes. It has the greatest and most constant value for lax plants; dense plants fluctuate markedly in height; pigmy plants, even when brought to flowering, are of little more than half the height of the dense. But height of plant may be said to have a constant association with ear density. Thus in what follows consideration may be restricted to this latter character.

§ IV. SUBSIDIARY TESTS.

From the facts, as recapitulated at the end of the preceding paragraph, it appears reasonable to assume the existence of a single factor *D* for ear density. The constitutions of the three genotypes may then be written lax = *dd* : dense = *Dd* : pigmy = *DD*. If normal segregation occurred at the gametogenesis of dense plants, the progeny ratio would be *dd* : *Dd* : *DD* = 1 : 2 : 1. Actually it is about 50 : 46 : 4 per cent. The divergence cannot be solely attributed to failure of seed to germinate or of young plants to survive, for that would require a mortality of 50 per cent. of the seed formed. Such a possibility is emphatically ruled out by the results of repeated survival counts. It must next be considered, therefore, whether a characteristic limited proportion of fertility among the gametes of dense plants affords an explanation. Since the ears of dense plants show no more than an ordinary proportion of sterile florets in comparison with wheat forms generally, the ♀ gametes must be regularly functional. With regard to ♂ gametes, the ears of lax (*dd*) plants being fully fertile, only the *D*♂ gamete can be supposed to suffer functional restriction. Hybridisations between lax and dense plants offer a suitable test of this supposition.

In 1923 extensive reciprocal crosses were made, the hybrid seeds from individual mother plants being grown as separate cultures. Of the cross *Dd*♀ × *dd*♂ there were 21 cultures with a total of 165 surviving plants

at the completion of ear emergence: for $dd\varnothing \times Dd\mathfrak{J}$, 12 cultures furnished 113 plants. Both populations contained lax and dense plants and, additionally, some stunted earless plants. These earless plants were of the kind familiar in selfed-dense progenies, and were therefore, as in those progenies, regarded as impoverished members of the dense genotype. It was quite certain that neither of the crosses resulted in the production of a single bushy pigmy (DD) plant. Nothing could be deduced from the lax : dense ratio in individual cultures which comprised only from 4 to 16 plants each. The average population values for combined cultures derived from the reciprocal crosses must therefore be used. These are set down side by side with the progeny ratio for self-fertilised dense plants. For this last named, the 1922 values are given: they, as described, fairly represent the order of the values obtained in the four years of investigation.

	Case (α)	Case (β)	Case (γ)
Parentage	$Dd\mathfrak{J} \times Dd\varnothing$	$Dd\mathfrak{J} \times dd\varnothing$	$dd\mathfrak{J} \times Dd\varnothing$
Gametes	$x.D \quad 1D$ $1d \quad 1d$	$x.D \quad 1d$ $1d$	$1d \quad 1D$ $1d$
Progeny expected	$1dd : (1+x) Dd : xDD$	$xDd : 1dd$	$1dd : 1Dd$
Actual progeny per cent.	49.3 : 43.9 : 6.8	6.2 : 93.8	58.2 : 41.8

In this scheme, x is the proportion of $D\mathfrak{J}$ gametes which are functional. Case (α) presents an apparent absurdity for comparing the actual and theoretical values of $dd : Dd$, the proportion x is seen to be a negative quantity. As explained in § III (above) the most constant ratio was dd :total plants and remembering the great inconstancy of the proportions of Dd and DD plants, it is reasonable to determine x from the proportion of dd :total plants. This, however, gives a value of x less than one-tenth of that deducible from case (β). Again, the divergence from expected equality of the two genotypes in case (γ) is too wide to be of chance origin. No doubt therefore remains that the simple hypothesis of a low proportion of functional gametes of the $D\mathfrak{J}$ kind, is inadequate.

But since the hypothesis has certain *prima facie* recommendations, the objections to it must be examined. The most apparent is inequality of the two genotypes in the straightforward case (γ). No linkage or other basis for inequality in the numbers of D and $d\varnothing$ gametes is suggested by facts, so that the only possibility appears to be a difference in germination or survival rates between the zygotes dd and Dd . Germination and survival data must therefore be examined.

On ear-forming dense (Dd) plants, shrivelled grains occur with about the frequency common to similarly grown forms of *T. vulgare* in general. All the grains look alike though, of course, they are genetically of three

kinds. But the apparent maternal nature of grain inheritance seen in all heterozygous wheat plants is a familiar mystery. Laboratory tests show that grains from both lax- and dense-eared plants have a germination of nearly 100 per cent. Survival data for ordinary outdoor sowings may be thus summarised for survival up to the end of normal ear emergence in early July:

Case	Parentage	Genotypes in the Progeny	Survival per cent. of Seed	No. of years of observation
(α)	$Dd\delta \times Dd\varphi$	$dd \ Dd \ DD$	73	4
(β)	$Dd\delta \times dd\varphi$	$dd \ Dd$	78	1
(γ)	$dd\delta \times Dd\varphi$	$dd \ Dd$	67	1
(δ)	$dd\delta \times dd\varphi$	dd	78	4

Assuming the survival of dd (lax) zygotes to be, as in case (δ), always 78 per cent., the calculated survival for Dd zygotes is from case $\beta = 78$ per cent. and from case (γ) = 56 per cent. To this incongruity similar computations will be found to add others. Thus zygotic survival data merely intensify the confusion they were employed to clarify.

A dilemma is only too patent. No hypothesis can be based on genotypic ratios without correcting these for specific zygotic survival rates; and the survival data themselves show disaccord. Thus after four years of observation on considerable numbers of plants, there is still no explanation for a phenomenon which, difficult to trace out in certain respects, is yet outstanding and defined in general form. In final recapitulation it may be thus described.

A dense-eared wheat, of which the precise origin is unknown, gives when self-fertilised three quite distinct genotypes. These may be called lax, dense, and pigmy. Their ear density increases in the order named, their plant height in the reverse order, the association between these two characters being specific and constant. Lax plants breed true and are of normal behaviour in general. Dense plants, besides showing segregation at gametogenesis, fluctuate markedly in height and general development. The pigmy plants are self-sterile, very fluctuant, and produce ears only under special growing conditions. While the precise survival rates and inter-proportions of these genotypes in dense-plant progenies are uncertain, they are unquestionably entirely different from expectation based on simple segregation of a single factor.

§ V. GENERAL CONSIDERATIONS AND EVIDENCE FROM OTHER INVESTIGATIONS.

In what has been described appears to lie a striking but obscure phenomenon. Other cases, similar in leading features, have from time

to time been recorded. To gather together the established general facts it is desirable briefly to review the more important related cases.

In animals, unusual segregation ratios found in mice by Cuénot and Castle, and in certain *Drosophila* cases by Morgan, have led to explanations involving non-functional gametes and non-viable zygotes. But it is in plants that evidence is most abundant. In them, for some reason, the phenomenon is usually, though not invariably, associated with dwarfness. Dwarfness, of itself, is an interesting characteristic. It may appear in self-fertilised stocks or in hybrid generations, and its inheritance may be simple or marked by variable abnormality. Here the matter needs no further consideration for its own sake. Waldron(4) has written a useful and succinct review of dwarfing as recorded in maize, beans (*Phaseolus*), wheat, and oats.

Among the strictly related cases, that of Vilmorin(1) is classical. Its substance has been given in § I above. Despite a general agreement between Vilmorin's results and those recorded in this paper, there is an important point of difference. With plant-height as diagnostic character a ratio of about 1:2.5 was found. But classifying by density of ear, which appears to have a regular association with plant-height, the corresponding ratio was roughly 1:1. Vilmorin made actual measurements of height but found—not unnaturally—that these sometimes falsified the classification. In this appears to lie the sole explanation of the difference between the two results: it is not wholly satisfactory. Vilmorin always had in mind the possibility that his "ever-splitting" dwarf was a heterozygote, its progeny type-ratio being disturbed by non-survival of one homozygote. Further, he hazarded the opinion that the dwarf arose by natural crossing. If these views are correct, the parents of this crossing remain a subject of speculation. Subsequent evidence—Vilmorin's own results and §§ II and III above—suggests that the bushy pigmy must have been one parent and the normal lax or tall the other. But this is highly improbable, for the bushy pigmy, so far as is known, was observed in ear for the first time in 1924.

Waldron(4) found dwarf plants in the progeny of the wheat cross, Marquis \times Kota. It is important to note that these wheats both belong to *Triticum vulgare*. In F_3 the segregation ratio of dwarf to normal displayed widely different values in individual families. All cases of this kind admit of two forms of tentative explanation. First, gametic defect, with or without restricted zygotic viability, may be employed. Alternatively, a multi-factorial explanation may be moulded into approximate agreement with the facts. Choosing the latter, Waldron

ingeniously obtains considerable success. But he expresses the final difficulty thus: "If...certain of the genotypes ordinarily producing plants of normal height become so modified that genes for dwarfness are changed to genes for normal and vice versâ, the facts may be accounted for." It is significant that from such carefully ordered investigation there arises the suggestion of factorial plasticity, and that simple gametic defect and zygotic non-viability find no place.

Other cases of dwarf plants which arise from *T. vulgare* \times *T. vulgare* hybridisations have been recorded, and Waldron's paper gives a résumé of the important ones. For two years the phenomenon has been observed at Cambridge in the progeny of Hard Federation \times Yeoman. The dwarfs were, as is usual, of the bushy pigmy type. Their proportion in the F_3 families of which they were members varied, and had for the combined families a value of $1:5 = \text{pigmy} : \text{remainder}$. Waldron's dwarfs produced seed, though not freely, while the Hard Federation \times Yeoman pigmies came to ear only under special conditions of growing. The ears bore no seeds.

An unusual occurrence of the sterile, bushy, pigmy plant is to be found in an instance described by Miyazawa(7). A 6-row and a 2-row barley were crossed and the F_1 then crossed back on to the 2-row. In the resulting progeny was one dwarf plant. Subsequent investigation showed this to be a heterozygote giving, when selfed, normal : dwarf (like the original dwarf plant) : sterile dwarf (bushy pigmy) = $1:2:1$. But the sterile dwarf was so susceptible to the influences of winter that in winter-sown cultures no plant of it survived. Here there are three features of interest. All cultivated barleys have seven haploid chromosomes, and thus the case is analogous to sterile dwarf occurrence in crosses between wheat forms of the same species. Next is to be noticed the complete susceptibility of the sterile dwarf to the influences of winter. Finally, and in relation to other dwarf cases most noteworthy, is the perfect simplicity of the segregation.

Schegalow(8) encountered giants instead of dwarfs in a pure line of oats. These appeared in the progeny of certain plants, found in field plots, which behaved like heterozygotes. Under xerophytic conditions the giants set a few seeds which grew true to giant type. Giants arising and behaving in fashion analogous to the dwarfs of other cases and in pure line material, make this instance of outstanding importance. Possibly a natural hybridisation was the origin, but of this there is no evidence. No difference in chromosome numbers was observed between giant and normal plants.

An ever-splitting barley, giving normal, ever-splitting, and dwarf has been described by Hallqvist(5). It arose in one of six strictly comparable F_2 families. The ratio dwarf : remainder fluctuated widely among individual F_3 families derived from F_2 ever-splitting plants. For these families combined it had a value suggestive of 1 : 3, but always showing a defect in the proportion of dwarfs. Tests of zygotic viability lent no support to the idea that this might be a factor in the production of irregular ratios. Test hybridisations of normal and ever-splitting gave results conformable with a hypothesis of an effective shortage of one male gametic type. The number of observations, however, was small. Certainly it was too small to settle the constancy or inconstancy of the predicated gametic deficiency which is a point of cardinal importance. The dwarfs formed no ears. It is noteworthy that a series of dwarfs was found in most of which chlorophyll-deficiency was characteristic. That inadequacy of evidence from check hybridisations leaves a distinct lacuna in this interesting body of proof of gametic deficiency is, perhaps, not an exaggerated comment.

It is, perhaps, in the instance reported by Åkerman(6) that the closest approach to an acceptable explanation is made. He discovered, in a commercial wheat, one plant abnormally tall, lax in the ear, and with peculiar chaff. This he called a speltoid-heterozygote, for its progeny contained normal (original stock type) : speltoid-heterozygote : a bearded speltoid type with very lax ear and of weakly constitution. The type ratio was subsequently shown to be 1.57 : 2.14 : 0.29. It is noteworthy that he also found "speltoid mutants" in a number of other wheats, some commercial and some of recent hybrid origin. A theory of effective shortage of one type of male gamete at gametogenesis of the heterozygote was propounded. Certain test hybridisations supported it closely, others did not: and between the results of two years were divergencies. To meet these difficulties three facts are put forward. Natural crossing was known to have occurred in a proportion of cases: the speltoid bearded genotype was not fully hardy, though the precise influence of this was not determined: and difficulty in emasculation of ears with speltoid chaff was naturally suspected as a source of error in test hybridisations. While the leading conclusions are convincing, doubt must surround the constancy of the results from year to year and case to case. In seeking the ultimate cause of the ever-splitting phenomenon in general, this inconstancy must always be heeded.

Enomoto(9) found in *Portulaca* an ever-splitting form of which the genetic behaviour showed features of peculiar interest. One homozygote

of the selfed-mutant progeny was considered to die almost as soon as formed. A lethal factor invariably fatal in the homozygous condition was postulated as the cause of this. No evidence of marked fluctuability in the mutant is recorded, but for germinable seeds set by the mutant the juvenile mortality was 17 per cent., in contrast with 4 per cent. for seeds of the original form among which the mutant appeared.

Other examples such as rogue-producing peas [Bateson and Miss Pellew, Blaringhem, and Brotherton] are not in sufficiently close relation to the special field of discussion to be noticed in detail.

From these collected cases, the widespread occurrence of "ever-splitting" is patent especially in cereals. The evidence of §§ II—IV (above) suggests the phenomenon may take a very recondite form. It is therefore desirable to set out the salient features of this particular case together with the facts from other cases relating to them. These features are:

(i) *The unknown origin of the ever-splitting form.*

As has been shown, Vilmorin's original heterozygote (dwarf = Dd) cannot well be regarded as having arisen from a crossing followed by normal segregation. In the cases described by Waldron, Miyazawa, and Hallqvist the abnormally segregating form arose in the gametogenesis of an artificial F_1 plant. Three other cases—Schegalow, Åkerman, and Enomoto—had no proved connection with hybridity, but natural cross-fertilisation must be suspected in the second of these. On the whole then, the origin of these abnormal plants is to be regarded as mutational, hybridisation being sometimes an associated, and perhaps an essential circumstance.

(ii) *Occurrence of three genotypes in the progeny of selfed dense (Dd).*

This established fact has its counterpart in all the cases considered. It is to be presumed, therefore, that the mutational change always affects only one gene: or if more than one, then the chromosome concerned rarely or never displays crossing-over.

(iii) *Inconstancy of the genotype-frequency ratio $DD : Dd : dd$.*

This is a leading feature of all the examples save those of Enomoto and Miyazawa. That the latter should be exceptional is remarkable, for the form and sterility of the one homozygote irresistibly suggest some fundamental in common with the other examples.

(iv) *Low survival rate of two genotypes (Dd and DD).*

In the case described in this paper survival rates are characteristically

low. Hallqvist and others found them to play no leading part in the explanation. But in all cases—even in Enomoto's *Portulaca*—there is some evidence of a difference in survival between normal and dwarf. Thus one effect of the mutational change appears to be lowered viability of at any rate one of the resultant types, as well as some degree of sterility.

(v) *Marked general fluctuability of the dense genotype (Dd).*

Most of the records place either little emphasis on fluctuability or leave it unmentioned. It deceived Vilmorin, and was a most perplexing feature of the inquiries described in the body of this paper. Possibly it is to be associated with survival rate: it cannot be ignored.

(vi) *Abnormally low proportion of one type of ♂ gamete (from dense = Dd).*

In the case concerned, as in those of Hallqvist and Åkerman, this hypothesis is strongly supported. But in no investigation is the proportion soundly established. Possibly, as the inconsistencies described in § IV suggest, it is an inconstant proportion. As here used, proportion refers to ♂ gametes effecting fertilisation. It is noteworthy that characteristic sterility in wheat inter-species progenies is traceable to defective ♂ gametes, the female gametes being always functional [Watkins(3), vol. xv]. Gametic defect should be demonstrable. Mere inspection of pollen grains reveals nothing in the particular case under discussion. In the other cases reviewed above no demonstration appears to have been attempted.

(vii) *The height-cum-ear-density gradation dd — Dd — DD.*

There is a suggestiveness in the association of plant height and ear density which was described in the concluding sentences of § III (above). Åkerman's case reinforces this. Now among wheat forms in general no such association is found. Thus, usually, the factors controlling plant height must be in a different chromosome from those controlling ear density. But in this case, ear density and plant height being definitely associated, the two sets of factors must stand in some special relationship. At gametogenesis the two sets behave as if in the same chromosome. There is thus a temptation to infer that the mutational change to which the ever-splitting form owed its origin, involved something equivalent to the welding of two chromosomes.

(viii) *The distinctive characteristics of the bushy pigmy genotype.*

Nothing marks out the phenomena described in the papers here reviewed more strikingly than the squat habit, the lateness, the feeble con-

stitution, and the high or complete sterility, of the bushy genotype. This form occurs in progenies of unknown origin such as Vilmorin's dwarf, and in the *T. vulgare* \times *T. vulgare* crosses of Waldron and others. But in inter-species crosses, notably *T. turgidum* \times *T. vulgare*, it is no less characteristic. Thus its occurrence strengthens the link with inter-species crosses which partial gametic deficiency has already forged. It has been abundantly demonstrated that partial sterility of inter-species hybrids arises from chromosome irregularity. A difference in chromosome number between the parents of the inter-species cross is most familiarly the cause of such irregularity. But in Vilmorin's wheat, original chromosomal difference or other irregularity is suggested by none of the known facts. This is true also of the cases of Waldron, Miyazawa, and Hallqvist, where dwarf forms arose in hybrid generations. If then, similarity to inter-species crosses is to be pressed, another attribute must be added to the hypothetical mutational change in which ever-splitting forms arose. Some irregularity—possibly a change in chromosome number—must have come about. Schegalow found his partially sterile giant oat to have the same chromosome number as the normal stock in which it originated. But without a more precise knowledge of his material than the abstract [Schegalow (8)] affords, it is not possible to determine the bearing of this fact on the ever-splitting phenomenon.

Assessment of the features of this ever-splitting wheat, supplemented by comparison with analogous cases, leads to two final conclusions. The general phenomenon has not yet been accurately described, or its constancy determined. Further, there is reason to suspect some chromosomal irregularity as the basic cause. This, together with the inconclusiveness of the best essays, points out the future pursuit of the problem as for the cytologist.

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INHERITANCE IN WHEAT.

II. *T. TURGIDUM* \times *T. DURUM* CROSSES, WITH NOTES ON THE INHERITANCE OF SOLIDNESS OF STRAW.

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(With Two Text-figures.)

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INTRODUCTION.

T. turgidum and *T. durum* both have 14 chromosomes and therefore intercross without sterility. Of their many botanical and economic differences endosperm texture is the most emphatic and most important. Endosperm inheritance in wheat has, in past experiments, appeared to be maternal, and this gives the *T. turgidum* \times *T. durum* crosses a critical genetic interest. Economically they are no less interesting. Rivet wheat, the only English form of *T. turgidum*, is valuable as a heavy yielder, but its endosperm is unsuited to bread making. Its crosses with the bread wheats (*T. vulgare* = 21 chromosomes) are attended by so high a degree of sterility as to hold doubtful economic promise. Rivet derivatives must consequently be sought in crosses with *T. durum*.

The results to be recorded bear mainly on endosperm inheritance, but simpler morphological characters were also observed. Their intrinsic interest is slight, but in the cereals groups of "genetically inseparable" characters are not infrequent. As a principle in cereal genetics, therefore, no character should be investigated alone. The inheritance of solidness of straw is one of the features of these *T. turgidum* \times *T. durum* crosses, but some additional evidence from other crosses is included.

§ I. THE PARENTS AND THE HYBRID GENERATIONS.

The standard account of the two species is to be found in Percival(3). For the actual parental forms the following are the salient features:

<i>T. turgidum</i>	<i>T. durum</i>
Tillers fairly well.	Tillers badly.
Straw tall, whippy, and solid at the neck.	Straw $\frac{3}{4}$ height of <i>T. turgidum</i> and hollow.
Paleae awned.	Paleae awned.
Ears long and heavy since number of grains per spikelet and of spikelets per ear are high. For these reasons also the ear is "square" in cross-section.	Ears correspondingly smaller and of an "oblong" section.
Glume sharply keeled and with symmetrical, bow-shaped margin: pubescent: becoming blackish-grey at maturity.	Glume similar but shape of margin less symmetrical: less pubescent: blackish colour at maturity very fluctuable.
Grain blunt at apex: back high but roundly arched: furrow open (Fig. 1).	Grain more pointed: back humped rather than arched: furrow compressed (Fig. 1).
Endosperm soft (test by biting): very white: starchy.	Endosperm very hard: clear and vitreous: fluctuant grains may be semi-starchy but even these are hard.
Slightly susceptible to <i>Puccinia glumarum</i> .	Much more susceptible.

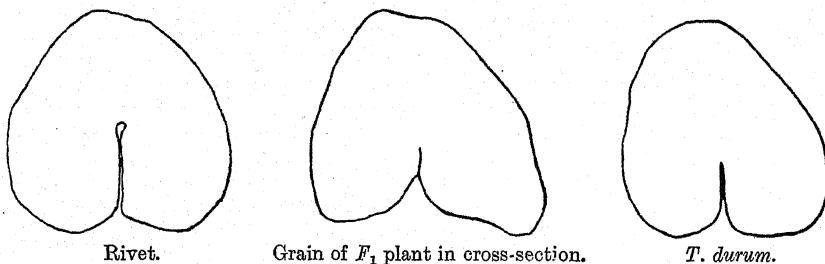


Fig. 1.

The F_1 were vigorous plants. They were taller than either parent, had larger ears, and larger grains. Most of the glume and other detail-characters were parental blends, but a strong general resemblance to *T. turgidum* resulted from the high "set" or number of grains in the spikelet. Grain form was distinctive. The size considerably exceeded the larger grained parent: there were, unmistakeably, the "hump" and the compressed furrow of *T. durum*. Two other *T. durum* characters were completely dominant—the vitreous texture and extreme hardness of the endosperm. In no particular, however small, were significant differences found among the grains borne by the F_1 plant. Thus in circumstances ideal for its appearance, evidence of the hybrid nature of the endosperm could not be traced. Of the significance of this more is said later, in § III.

Complete F_2 's were grown for three of these crosses: one of these

was further carried on to a complete F_3 : from the other two, partial F_3 's were raised. In every case, the F_2 at first sight appeared to show no certain evidence of segregation. Soon after fertilisation, however, it became apparent that there were differences in time of ripening, and in susceptibility to *Puccinia glumarum*. These differences, however, were not sufficiently distinctive to promise genetic results of much precision. Observation on these characters was therefore abandoned. A considerable number of characters was investigated in the ripe material and the results are now to be discussed. In all three crosses the same form of *T. turgidum* was used; the *T. durum* form being different in every case.

§ II. EAR AND GRAIN CHARACTERS.

The progeny of a single F_1 plant is called an F_2 family. In all the crosses, F_2 families have been kept separate, as have the sets of data derived from them. For economy of space, family data are not given, but simply the data for the complete F_2 . But the values of all inheritance ratios have been tested, family by family, against the calculated errors of sampling. Every complete F_2 ratio given below has proved reliable on the basis of this test.

(a) Shape of Cross-section of the Ear.

All three forms of *T. durum* used in these crosses have laxer ears than the *T. turgidum* parent. In addition the *T. durum* spikelet has a smaller "set" or average number of grains. Consequently the *T. durum* ears appear oblong in cross-section, and the *T. turgidum* appear square. The difference may be measured either in terms of cross-section dimensions, or of average number of grains per spikelet, but neither measurement is satisfactory. Some square ears become altered to oblong towards the tip, and it proved impossible to specify the region in which cross-section should be measured. Number of grains per spikelet proves impracticable because even the parental distributions overlap considerably. In this, as in many such cases, eye-judgment of shape proved to be best.

In F_2 .W 27 square, intermediate, and oblong ear-sections were recognisable. Their ratio was 116 : 244 : 108. This is acceptable as a 1 : 2 : 1 ratio. It was more difficult to separate intermediate from oblong than from square, and the ratio square : remainder = 116 : 352, which is very close to 1 : 3. For F_2 .W 26 the only consistent grouping was square : remainder. Its value was 51 : 159, which confirms the unifactorial result found in F_2 .W 27.

Ear shape is compounded of ear density and set, or average number of grains, in the spikelet. Its unifactorial inheritance suggests that its two component characters are "genetically inseparable." This may mean that they are very closely linked, or that they are physiologically associated. To decide between these alternatives would require much more extensive data. Physiological association of characters has often been suggested in the cereals (*e.g.* Engledow (1), pp. 123-5). Economically the simple transmission of a difference in set of the spikelet is important, since spikelet size is a factor of yielding capacity.

(b) *Pubescence of the Glumes.*

Both parental forms are pubescent. In *T. turgidum* the hairs are long and evenly distributed but in the forms of *T. durum* used they are shorter, and more abundant on the keel and outer nerve than elsewhere. It proved impossible in F_2 to do more than discriminate between the *T. turgidum* type of pubescence and the remainder. This gave a ratio of 354:114, which agrees satisfactorily with unifactorial expectation. In some *T. vulgare* crosses (Howard, H. and G. (5), pp. 7-15) long and short hairs proved to be independently inherited: in a *T. polonicum* \times *T. durum* cross (Engledow (1), pp. 125-6) a connection was traced between pubescence and glume length. Neither of these phenomena appeared in these *T. turgidum* \times *T. durum* crosses.

(c) *Grain Size.*

The parental grains differ little in size and weight. But it was clear from the F_2 and F_3 that size factors were segregating. Preliminary measurement showed that classification on a metrical basis would be impossible. In an earlier cross between parents with grain length 7.7 mm. and 10.2 mm. respectively the F_2 grain length had a unimodal distribution (Engledow (1), pp. 121 (γ) and § IV). This could not be dissected into components statistically. Dissection was made possible by the fact that glume length was an index to grain length. But in these *T. turgidum* \times *T. durum* crosses it was clear that no such index relation subsisted. Consequently there remained only the possibility of judging grain size by eye. This gave inconsistent results on repetition. All that can be done therefore is to illustrate the effects of size-factor segregation by the following data for a few outstanding types. The numbers in full type are average grain lengths in mm.: those in *italics* are average grain weights in grammes.

	<i>T. turgidum</i> × <i>T. durum</i>	
P_1	6.21	7.00
	0.0445	0.0449
F_1	6.95	
	0.0554	
	<i>T. turgidum</i> types	<i>T. durum</i> types
F_2	5.39 to 7.33	5.13 to 7.50
	0.0252 to 0.0595	0.0216 to 0.0642

The extremes of length and weight exceed the parental ranges in both directions. It is believed, though there is no actual proof, that among the large grain segregates the vitreous endosperm and the shape of *T. durum* predominated.

The facts, though incomplete, present a striking contrast to the *T. polonicum* × *T. durum* cross already mentioned (Engledow (1) and (2)). There, grain length and glume length appeared both to be governed by a single factor. In *T. turgidum* × *T. durum* it is clear that grain and glume length have separate factors, and that there are at least two grain length factors. Thus *T. turgidum* must be very different genetically from *T. polonicum* and *T. durum*, although all three have 14 chromosomes. The segregation of very large grain wheats is noteworthy, for large grains are, in general, commercially desirable. Grain size inheritance is an attractive genetic problem and of great importance in plant breeding, but the experience here related, with that of other investigations, makes it clear that absolute measurements will not serve to specify grain size for genetic purposes. Ratios may be of more value. For example, grain length: grain width might be suitable.

(d) Colour Inheritance.

The grain of *T. turgidum* is, agriculturally, "red." Its testa carries a red pigment, but this is seen through the pericarp and against an intensely white background of endosperm. The actual appearance is therefore not red but a light red-brown. In the *T. durum* used the grain has a clear amber colour. This is the colour of the clear, vitreous endosperm, for the testa carries no red pigment. The grain of the F_1 plant is definitely red, a result produced by the red testa pigment of *T. turgidum* against the dominant vitreous endosperm of *T. durum*. Various colour shades were found in F_2 . Red like F_1 and red-brown like *T. turgidum* were common: the light amber of *T. durum* was rare: doubtful intermediate shades also occurred. On some single ears the grains fluctuated considerably. Reliable classification was impossible. Grains with vitreous endosperm could readily be classified: it was im-

possible to appreciate shades of testa colour against a starchy endosperm.

With favourable material grain colour inheritance has in a few cases been successfully traced (*e.g.* Nilsson-Ehle(6), pp. 66-75). There have been many failures, and it seems probable that they are attributable to the influence of endosperm texture.

Further study of colour inheritance in these crosses is desirable for an important technical consideration is involved. With appropriate baking Durum wheats can be used for bread, but it is necessary to bleach the objectionable yellow colour of the flour. The breeding of forms free from this colour hinges on the still intangible nature of grain colour inheritance.

(e) Grain Shape.

In this the parents display three differences (*vide* § I)—blunt or pointed apex: round or humped back: open or compressed furrow. Only one, round or humped back, had a demonstrable form of inheritance. For F_2 . W 27 round : humped = 123 : 356, an acceptable 1 : 3 ratio. The transmission of the other two differences was obscured by a curious shrinkage of the grain, suggestive of immaturity, but characteristic of *T. durum*. No satisfactory explanation of this curious feature has yet been found. In segregation it remains associated with the hardness of *T. durum*, but it is simulated in some soft-type grains by the impoverishment induced by *Puccinia glumarum* (yellow rust).

Brief mention may be made of two other characters. Susceptibility to *Puccinia glumarum* appeared not to be the unifactorial feature which has been demonstrated in *T. vulgare* inter-crosses. Among the F_2 plants and F_3 families were forms less susceptible than the *T. turgidum*, and others much more susceptible than *T. durum*.

Neither *T. turgidum* nor *T. durum* tillers well: the former is, however, certainly the better. Indifferent tillering may, indeed, be the supreme breeding limitation. Of all cereal characters tillering is the most fluctuant, and at present its mode of inheritance is quite unassailable. But it is believed that some of the F_3 segregates are homozygous for a higher degree of tillering than the *T. turgidum*. Abundant tillering is characteristic of F_1 and of F_2 forms heterozygous in a number of ear and grain characters.

§ III. ENDOSPERM TEXTURE.

Endosperm texture is the most important commercial character of the wheat grain. Its outstanding features in the three main cultivated

species must be considered. *T. turgidum* wheats have soft, white, starchy endosperms, of low protein content, and suited to unleavened biscuits rather than bread. In *T. durum* the endosperm is hard, clear, vitreous, rich in protein, and suitable for macaroni. Bread is made from the *T. vulgare* wheats. Their endosperm ranges from soft and starchy to hard and flinty. These extremes superficially resemble *T. turgidum* and *T. durum* respectively, but have entirely different baking properties. The flinty forms of *T. vulgare*, as they have a high baking quality, are known as "strong" wheats, the starchy being, correspondingly, weak.

Chemically, the endosperms of *T. turgidum* and *T. durum* are known to differ in protein content, but their other differences have not yet been disclosed. There is more knowledge of the *T. vulgare* wheats. Wheat protein or gluten consists of the two protein bodies, glutenine and gliadine. Woodman(4) has recently traced the difference between a strong and a weak *vulgare* wheat to a difference in the form of the glutenine, the gliadine being alike for both. On this may be founded a chemical interpretation of botanical differences for which wheat endosperm is very suitable material. If genetic factors for endosperm texture can be identified, this promising *liaison* between chemistry and botany may lead to a chemical interpretation of the nature of such factors. A demand for none but strong wheats in English agriculture is arising. This affords a further and insistent stimulus to genetic studies of the endosperm. At present, in genetics, as in commerce, endosperm texture can be judged only by eye.

The data to be given is from F_{1-2-3} . W 29 = *T. turgidum* (Rivet) \times *T. durum* (a Mesopotamian form). Parental endosperm differences, described in § I (*supra*), were, in some grains, obscured by fluctuation. This was carefully studied in preparation for corresponding difficulties in the hybrid generations. It was a rule to examine every grain of every plant. Classification was based on the characters vitreous or starchy, and hard or soft.

F_1 plants bore grains as vitreous and hard as the parental *T. durum*. All were alike, a fact discussed later in connection with maternal inheritance. In classifying the grains borne by the F_2 plants the following types of endosperm were recognised:

R = all grains like *T. turgidum*.

$R-$ = all grains like *T. turgidum*, save that there was a small vitreous "patch" in the starchy matrix.

RD = grains not homogeneous: some more starchy than vitreous: others more vitreous than starchy.

$D-$ = all grains like *T. durum*, save that there was a small starchy "patch" in the vitreous matrix.

D = all grains like *T. durum*.

In this frankly arbitrary classification, types R and D are distinctive. For $R-$ the resemblance to *T. turgidum*, is strong, but the constant appearance of the vitreous patch justifies a separate type. The same applies, *mutatis mutandis*, to $D-$ in relation to D . Fluctuant *T. turgidum* grains might be confused with $R-$ and, correspondingly, *T. durum* for $D-$. Difficulties are therefore to be expected in classification. Type RD is the most difficult. From the same ear may come grains predominantly starchy, and others predominantly vitreous. All had an inter-parental hardness. It was concluded that the plants were of definite genotypes peculiarly prone to fluctuation. This conclusion was reached after careful consideration, and with full regard to the fact that apparently different grains on the same ear might be evidence against the maternal nature of inheritance which the F_1 plants suggested. The frequencies of the types in F_2 were:

R	$R-$	DR	$D-$	D
21	18	57	15	37

No positive factorial scheme consistent with the F_3 data can be suggested for this distribution.

144 F_3 families were classified plant by plant as R , $R-$, DR , $D-$ and D . For the genetic classification of the F_2 progenitor plants the first step is the collection of F_3 families into groups. Now only nine families had grain of a single type. This was type D and as $\frac{9}{144} = \frac{1}{16}$ bifactorial inheritance is indicated: it was confirmed by general impressions as sorting proceeded. A two factor difference would give nine groups of F_3 families but the actual F_3 data did not fall naturally into nine groups. Arbitrary and necessarily somewhat speculative resolution of some of the F_3 groups is thus inevitable. But endosperm characters have a recondite nature, an undiscovered inheritance, and great economic importance. This appears to justify such a treatment of the incomplete results of what proved a rather arduous investigation. The groups of F_3 families were:

I. 9 families comprising 115 plants on all of which every grain was of D type.

II. 20 families in which only D and $D-$ plants occurred. The ratio was $D- : D = 66 : 201 \approx 1 : 3$.

III. 21 families all having DR , D and $D-$ plants. The ratio

$DR : \overline{D + D-}$ ranged from 1:1 to 1:4 in individual families, being, for the whole 96:210, possibly a 1:3 ratio.

IV. 9 families having the same grain types as III but the ratio $DR : \overline{D + D-}$ ranging from 1:6 to 1:18. The separation of III and IV is warranted by the form of the distribution, for III is a very compact group.

V. 34 families all having plants of types $R-$, DR , $D-$ and D , the proportions being 60:160:111:164, which gives

$$\overline{DR + R-} : \overline{D + D-} = 7:8.75 \simeq 7:9.$$

VI. 4 families containing the types R , $R-$ and DR in the proportion 20:15:12. This can be ascribed to no form of segregation and, in the light of parental behaviour, may well represent extreme fluctuation.

VII. 9 families containing R , $R-$, DR and $D-$ in the proportion 20:26:41:18.

There remained 38 families containing R , $R-$, DR , $D-$ and D . They fall into two groups.

VIII. 17 families in all of which $\overline{R + R-} : \overline{D + D-} > 1$ and for which jointly, $\overline{R + R-} : \overline{DR + D + D-} \simeq 1:3$.

IX. 21 families in all of which $\overline{R + R-} : \overline{D + D-} < 1$.

The grains of the F_1 plants show the *T. durum* endosperm type to be completely dominant. Moreover the proportion of parental *T. durum* forms is $\frac{1}{16}$ (Group I), and as a whole the F_2 and F_3 bear a stronger resemblance to *T. durum* than to Rivet (*T. turgidum*). This suggests the following constitutions:

$$\text{Rivet} = aa.bb : T. \text{ durum} = AA.BB.$$

The most reasonable identification of F_3 family groups with F_2 expectation then appears to be:

F_2 family group	No. of families in group	Presumed F_2 progenitor	No. of F_2 plants expected
I	9	$AA.BB$	9
II	20	$Aa.BB$	18
III	21	$AA.Bb$	18
IV	9	$aa.BB$	9
V	34	$Aa.Bb$	36
VI	4	$aa.bb$	9
VII	9	$AA.bb$	9
VIII	17	$Aa.bb$	18
IX	21	$aa.Bb$	18

This theory is supported by the F_1 plants and by the proportion $\frac{1}{16}$ of $F_3.D$ families. Groups I, II and V appear to meet theoretical re-

quirements fully: Groups III, IV, VIII and IX meet them acceptably. Groups VI and VII premise great fluctuability of the genotypes they represent in the scheme. This is not considered impossible. There may be other and more recondite endosperm factors, and their segregation might well vitiate the classification based upon *A* and *B* only. It is possible to aggregate the theoretical genotypes in accordance with the actual F_2 distribution, but this has no critical value. The progenies of individual F_2 plants best display the harmony of the F_2 and F_3 classifications. They show that some F_2 plants classed as *D* had both *D* and *D*- among their progeny: a few classed as *D*- had only *D* progeny and so on. The complete dominance of the *T. durum* endosperm makes some of these disharmonies inevitable; and further, in a badly matured *T. durum* sample, examined on the basis here adopted, some grains would have to be classed as *D*-.

A and *B* are regarded as factors producing separately a durum-like endosperm and jointly a true-durum one. No novel type is therefore to be expected in the segregates of the cross. And, as a fact, the grains of the F_2 and F_3 plants either resembled the parental forms or were intergrades. This closes the speculative possibility that forms like *T. vulgare* might result from combinations of the softness of Rivet and the high protein content of *T. durum*. Mere replicas of this investigation, even carried to F_4 and F_5 , would be unlikely to give more precise results. There must first be a study of endosperm texture to devise an adequate classification of types.

Since wheat endosperm is a triple fusion product, the grains should be a generation ahead of the plant which bears them. In earlier investigations (*e.g.* Engledow (1) and (2)) it proved necessary to ignore this, and to treat grain-endosperm and plant as effectively of the same generation. The same treatment was suggested here, at the outset, by the palpable homogeneity of the grains of the F_1 plants. This, with parental types so emphatically different, is strong evidence. It was fortified by an examination, grain by grain, of some 2500 F_2 and F_3 plants. Freeman (7), accepting the implications of triple fusion, has elaborated the data from his *T. durum* \times *T. vulgare* crosses accordingly. But it is a fair comment that the weakest part of his interesting demonstration is the failure to confirm in F_2 the differences he perceived among grains borne by the F_1 plant. He naturally experienced all the difficulties of fluctuation previously described: but it seems unwarranted to ignore the possibility of maternal inheritance when the available data lack precision.

An expectation of effectively maternal inheritance of wheat endo-

sperm may be theoretically deduced. In wheat, from fertilisation to maturity of the grain, is a period of only about two months. At maturity the endosperm, save for a few cells, is dead (Miss Brenchley (8), pp. 131-3). Factorially different endosperms on the same ear are nourished by a common food supply and the factors have less than two months in which to impress their characteristics. That such endosperms should appear palpably different seems, from this point of view, no more probable than that there should be marked differences between their accompanying embryos two months after germination. It is argued, in short, that effectively maternal inheritance is to be expected because the endosperm dies in a juvenile stage. It is not possible to examine this argument in the light of many other results. In maize, harmony with the implications of triple fusion appears to have been achieved. In *Pisum*, maternal inheritance of seed form was found in round \times indent but not in round \times wrinkled. There, however, seed form is determined not by dead endosperm tissue, but by the cotyledons.

§ IV. THE INHERITANCE OF SOLIDNESS OF STRAW.

In the graminaceous stem are two layers of ground tissue. The hypodermis consists of tough, partly lignified fibres, and within it is the pith. This is a broad layer of parenchyma whose cells dry and shrink before the plant matures. Most wheats have a central stem lumen whose diameter, like the width of the surrounding pith layer, varies from point to point of the length. The form of this variation characterises the different wheats. Some, along almost the whole stem, are solid with pith. Others are solid only in the top internode, and there are semi-solid gradations. Finally there are wheats with a large lumen in every internode, to which the pith makes a thin lining. These are called hollow straw wheats. The terms solid and hollow disclose none of these finer variations, but imply that solidness is primarily dependent on amount of pith. But diameter of straw and thickness of wall (hypodermis) also determine the degree of solidness. Complexities may therefore be expected in hybrid generations. Elaborate differences were actually found, and it proved necessary to base judgment upon the degree of solidness at only two points. One was in the "neck," at about three inches below the ear, the other at the base of the top internode, between which points was a gradation of solidness in all forms examined. It is now believed that the "lodging" of cereals is largely dependent upon the elasticity of the straw as distinct from its strength. Solid straw wheats commonly have great elasticity, and it is therefore desirable to

determine the mode of transmission of solidness in connection with the breeding of non-lodging forms.

Four crosses involving five parent forms are to be described. Fig. 2 illustrates the degree of solidness in the parent stems at the two selected points. Rivet and Polish clearly exemplify the influence of straw size on degree of solidness. The former has the narrower neck but at the base of the first internode is considerably the wider. Thus it is that in Rivet the neck is completely filled with pith but there is a lumen at the base, while in Polish the converse holds. In respect of degree of filling of the top internode by pith the parent forms are a graded series, the order being as in Fig. 2.

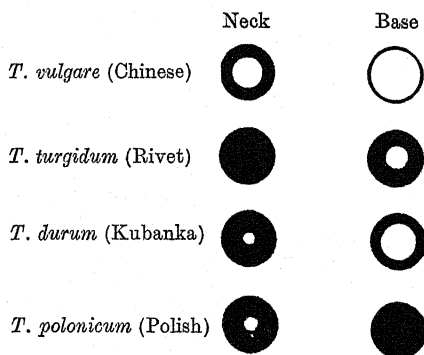


Fig. 2. Showing relative amounts of pith.

In Polish \times Rivet all the F_2 plants naturally had a very small lumen at the neck. Consequently they were classified on "lumen at the base." The types found were:—

- | | |
|---|--------|
| (α) Completely solid like Polish | = 244 |
| (β) Very small lumen | = 29 |
| (γ) Lumen about as large as in Rivet | = 120 |
| (δ) Almost as hollow as <i>T. vulgare</i> (see Fig. 2) | = 129. |

The occurrence of (δ), *i.e.* forms more hollow than even Rivet, suggests the operation of at least two factors. One of these might represent the greater solidness of Polish, the other the greater straw girth at the base of the first internode which characterises Rivet. It will be found that without unduly straining assumption, the actual proportions can be made approximately to fit such a theory. But the safest deduction is that hinging on the ratio (δ) : remainder, *i.e.* definitely hollow : remainder. Its value, 129 : 393, is about 1 : 3. The conclusion is that the Polish type of solidness is simply dominant to the Rivet

type but that factors for straw size, in segregation, produce secondary types.

Rivet \times Chinese gave the following F_2 :

(ϵ) Top internode solid along the whole length	= 16
(ζ) Like Rivet	= 64
(η) Small neck lumen: less solid than Rivet at the base	= 35
(θ) Like Chinese	= 37.

Here again is an extra-parental type, but definitely hollow: remainder = $\theta : \epsilon + \zeta + \eta = 37 : 115 \simeq 1 : 3$. Inheritance is thus unifactorial. The number of F_2 plants is small because of the partial sterility of the F_1 from a cross of 14×21 chromosomes. It is presumed that sterility had a non-selective incidence in relation to solidness.

Polish \times Kubanka proved difficult when attempts were made to recognise gradations of semi-solidness (Engledow(1), p. 126 and (2), p. 94). Here, finally, as in the other two crosses, certainty attached to no ratio other than hollow (as in Kubanka): remainder, the value of which was clearly proved to be $1 : 3$, the observations extending to F_5 .

Straw inheritance was also investigated in two of the Rivet \times *T. durum* crosses described in §§ I—III above. The two *T. durum* parents had straw more hollow than Kubanka but more solid than Chinese (*vide* Fig. 2). Classification based upon the lumen size at the bottom of the top internode gave:—

F_2 . W 26 hollow: remainder = $49 : 163$, a ratio whose divergence from $1 : 3$ may be attributed to errors of sampling.

F_2 . W 27, for some unknown reason, allowed of the recognition of solid (like Rivet): intermediate: hollow (like F_0 . *T. durum*). The ratio of these in every F_2 family and for the complete F_2 was statistically acceptable as $1 : 2 : 1$.

In these crosses therefore unifactorial inheritance is established. The greater amenability to grouping of F_2 . W 27 is interesting evidence of the play of other straw characters upon degree of solidness.

As Fig. 2 suggests the parent forms may be ranged in order of solidness thus: (1) Polish, (2) Rivet, (3) Kubanka, (4) Durum parent of Rivet \times Durum, (5) Chinese. That unifactorial differences have appeared in (1) \times (2), in (2) \times (5), in (1) \times (3), and in (2) \times (4) is suggestive of "multiple allelomorphism." But having regard to the influence of other straw characters on "solidness," the safest conclusion appears to be merely that a greater

amount of pith is simply dominant to a lesser amount. In connection with the breeding of non-lodging wheats, and to interpret the complexities found in Polish \times Rivet and in Rivet \times Chinese, it will be desirable to investigate other straw characters such as girth and thickness of wall. Wheats like Indian Runner and Huguenot, solid in every internode and of great elasticity, may prove both interesting and economically important.

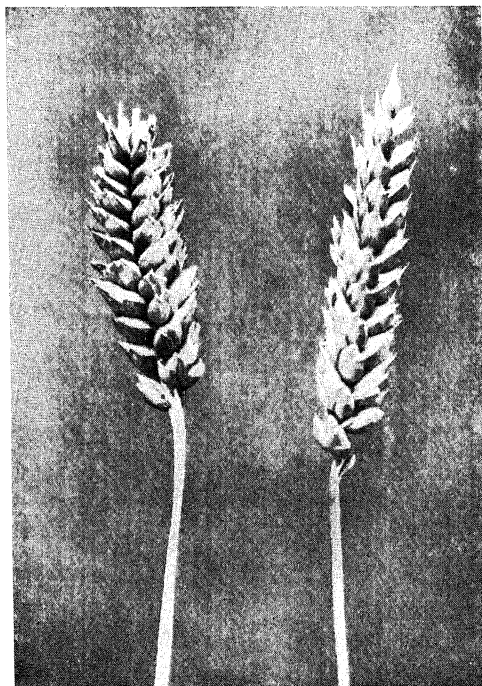
Solidness of straw appears to be neither linked to, nor genetically inseparable from, any other character of the wheat plant.

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(a)



(b)

(c)

(a)=one of the bushy pigmy plants, flowering under the special conditions described in the text. The shortness and great density of the ear is noteworthy.

(b)=the ear of a normal "dense" plant.

(c)=the ear of a "lax" plant.

"SOMATIC SEGREGATION" IN DOMESTIC FOWL.

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(With One Colour Plate.)

FROM most other domestic birds and from mammals domestic fowl differ in the absence of a kind of coloration called "piebald." When we say "piebald" we mean the presence of more or less irregular spots on the skin with no pigment; while the derivatives of the skin—feathers and hair—are upon these spots also deprived of pigment. Among mammals we know piebald horses, asses, cows, deer, goats, sheep, swine, dogs, cats, rabbits, guinea-pigs, rats, mice, wild bisons etc. Among birds we find piebald geese, ducks, pigeons, canary-birds, sometimes sparrows, etc.

The study of fowl-hybrids of the race Plymouth Rock with other races showed, however, the presence of coloration not only resembling the "piebald" condition, but also serving to explain its origin.

As is known, the race Plymouth Rock has barred feathering, each feather being transversely striped black and white (Pl. II, fig. 1). The number of such stripes is usually five black stripes and five white ones, but this number may vary more or less, depending upon the size and character of the feather, and upon other reasons. In the "hackle" of the cock, and especially in the long tail feathers, the number of stripes can greatly increase.

When carefully examining the feathering of Plymouth Rock hens, one notices on most an occasional feather, differing in colour from other feathers. Generally such feathers are quite black. Sometimes such occasional feathers are grouped. Once we happened to observe in the hackle of a hen six black feathers tightly grouped together.

Still more often it has been noticed, that not the whole feather but only a part of it is of exceptional coloration. In most cases the distal end is normally coloured, the other half being quite black; sometimes the proximal end is striped on one side.

Such exceptional black feathers occur almost always on hens only. In homozygous Plymouth Rock cocks these abnormalities are not known to us, but only a few cocks have yet been studied.

We are inclined to consider the occurrence of these exceptionally

coloured feathers as a manifestation of an odd "piebald" coloration, for the reason that in other animals the piebald coloration is also not limited by the distribution of coloured and uncoloured spots. For instance in mammals another kind of piebald coloration often occurs in which red spots are spread on a black ground. These spots are irregular in the same degree, and often not symmetrical; their variability is also very marked as is that of black and white piebald coloration, and there is no fundamental difference between the two varieties of piebald coloration. There are black and red rabbits, guinea-pigs, mice, dogs, cats, gold-fishes, etc.

In dogs we have also a third kind of piebald coloration in which, on a uniformly coloured ground, are spread piebald spots, where coloured hairs are mixed with white ones, as in "silver" rabbits.

Here we have different kinds of piebald coloration. However, the following case of piebald cats leads us to another distinct kind of "piebaldness." It is well known that white blue-eyed cats are deaf. Genetical study established that this deafness is in some way correlated with the blue coloration of the eye, and this last is itself part of the piebald coloration. For if a white piebald spot happens to fall upon the region of the eye, the corresponding eye turns out to be blue, instead of the usual yellow. Such piebald eyes also occur in men, dogs, rabbits, etc. But with cats there is an additional complication, and the ear on the side of the blue eye is deaf.

It is therefore possible to point out the essential character of the piebald condition, viz. the mosaic distribution of some features, not only of the skin coloration, but also of the hair, or of iris coloration, or of the composite elements of hearing.

Such an explanation of the piebald condition might be adapted to facts we have observed in Plymouth Rocks. Here we have to deal with the irregularities in the distribution on the body of some elements of coloration, forming the irregular regions, the size of which varies from part of a feather to a group of many neighbouring feathers. These spots are distributed at random and asymmetrically.

Let us follow the analysis of this character. For this purpose it is necessary to notice the genetical elements of the Plymouth Rock coloration.

The Plymouth Rock coloration differs from the wild fowl coloration by the following principal genes¹:

- (1) *Tifa*-gene, determining the increased development of the black

¹ Our genetical nomenclature is as follows. Either gene is symbolised with a number similar to the principles of decimal bibliographical classification. The genes for coloration

pigment, and in some combinations capable of giving the self-black coloration of Minorca, Langshan, etc.

(2) *Trage*-gene, determining the transversely striped distribution of pigments in the feather; in the presence of the *tifa*-gene the transverse stripes can be black and white, in the absence of same—red and white, or brown and white, etc.

(3) *Tuge*-gene, *i.e.* the gene for the silver coloration. Normally this gene is not to be observed in the Plymouth Rock coloration, and the typical coloration of the Plymouth Rock can exist without it. That it cannot be observed is chiefly owing to the *tifa*-gene. Nevertheless, in most Plymouth Rocks there is a *tuge*-gene. It suppresses the development of golden elements in the coloration. We can usually notice that fowls with *tifa* and *trage* without *tuge* (*i.e.* in the presence of its recessive allelomorph *atuge*), have some golden edges or spots in the hackle, and probably owing to these golden spots, spoiling the standard of the race, the fowl-breeders have conserved the *tuge*-gene in the composition of the race.

These last two genes *tuge* and *trage* are localised in the sex chromosome, and *tifa* in an autosome. Therefore on crossing a Plymouth Rock hen with a "common" black-breasted red cock all the sons show the Plymouth Rock coloration, and all the daughters a pure black one, inheriting from the mother the *tifa*-gene, which is localised in the autosome, while they cannot get the *trage*-gene, localised in the sex-chromosome. In such a way the hens lose, so to speak, the *trage*-gene and turn out pure black.

We suggest that it is possible to consider the "exceptional black" feathers in a fowl as having lost for some reason the transversely striped design, thereby revealing the latent black coloration.

belong to the 8th group, the ground genes for colour belong to the 81st group, the genes for albinism to the 811th group, and so on. By aid of a special table these numerical symbols are transferred in short words. For a recessive allelomorph to this word is added "a": *tedu* is the gene for colouring, *atedu* is its recessive allelomorph, the gene for albinism, and so on. In this paper the following genes are mentioned:

<i>tedu</i> -gene (8114)—the ground-gene for colouring;	<i>atedu</i> —albinism.
<i>tifa</i> -gene (8215)—the "melanic"-gene, see p. 34;	<i>atifa</i> —the normal condition.
<i>tode</i> -gene (8311)—the "inhibitor of black pigment in Blue Andalusian";	<i>atode</i> —the normal condition.
<i>todi</i> -gene (8312)—the inhibitor of most colouring, the gene for epistatic white in White Leghorn;	<i>atodi</i> —the normal condition.
<i>tuge</i> -gene (8721)—the gene for "silver," see p. 35;	<i>atuge</i> —the gene for "gold."
<i>trage</i> -gene (8521)—the gene for barred-condition;	<i>atrage</i> —its absence.
<i>trakla</i> -gene (8535)—the gene for light-coloured marking and light shaft;	<i>atrakla</i> —its absence.

This point of view is decisively confirmed by considering the hybrids of the first generation from Plymouth Rocks with other races. We have had some hybrids between Plymouth Rocks and Orloff fowls, and also English-game race: the last two races are alike in coloration, the above-mentioned genes, *tifa*, *trage* and *tuge* being absent. The cocks have a black breast, and a more or less bright red back, hackles and saddle.

When we designate the genes *tuge*, *trage* and *tifa* by corresponding symbols Tu, Tg, Tf, and their recessive allelomorphs by tu, tg, tf, we get for the hybrids of first generation Plymouth Rock-Orloff the following formula:

$$\text{Chromos. I. } \frac{Tu \ Tg}{tu \ tg} \quad \text{Chromos. II. } \frac{Tf}{tf}.$$

Careful observation has shown the presence of exceptional feathers in such F_1 cocks. Most of these exceptional feathers proved to be black with red borders (cf. Pl. II, fig. 2). We can get the same exceptional feather, if we add to the Orloff's coloration the sole gene *tifa*: this gene would darken the middle part of the feather to an intense black, leaving its borders red (by simultaneous action of other genes which we do not mention just now).

Therefore, if the exceptional feathers were potentially black and, as it were, removed from the influence of the *trage*-gene, it is obvious, that these feathers are at the same time removed from the influence of both of the genes, which are localised in the sex-chromosome: they are neither transversely striped nor silvery.

In the same cock (N 2344) we have observed in small numbers also other exceptional feathers: white, black and so on, but we will speak of them later on. We would emphasise here that almost all exceptional feathers in Plymouth Rock-Orloffs are black with red borders.

This fact is to be explained as follows: since the exceptional feathers are simultaneously exempted from the influence of two genes, which possess a single common feature, namely being localised in the same chromosome, the whole phenomenon can be explained by the supposition that *these feathers are exempted from the influence of this chromosome*.

In the descendants of these hybrids, which are heterozygous in relation to *tuge* and *trage*, a segregation is of course to be observed. Omitting the possibility of crossing-over, which we shall examine further on, we can say that some of the offspring get both genes, included in a sex-chromosome, while others do not acquire this particular chromosome, and are at once deprived of both genes.

Thus the simplest suggestion would be, that, for some reason or other

during development, the exceptional feathers lose one of their sex-chromosomes, and if this chromosome turns out to be a chromosome bearing *tuge* and *trage*, the feather becomes the same as in descendants completely deprived of this chromosome.

Thus in the development of exceptional feathers there would appear to be some form of somatic segregation whereby some feathers get one set of genes and other feathers another set; and *as in the normal segregation the genes tuge and trage, localised in the same chromosome, show linkage, this same linkage is also to be found in the "somatic segregation."*

I offered such an interpretation of this appearance of exceptional feathers at the colloquium of the Institute of Experimental Biology in March, 1923. At the same time I pointed out the method of verifying that suggestion. If the character of exceptional feathers depends upon the chromosomal linkage of the genes *tuge* and *trage*, it ought to be altered by the change of the hybrid structure of the bird; as, for example, in the case when the genes *tuge* and *trage* are localised not in the same chromosome but in two homologous ones. The normal feathers containing the two chromosomes would have in both cases the same appearance, but the exceptional feathers should look quite different. If the chromosome bearing *tuge* vanishes, the feather becomes *tu Tg*, *i.e.* transversely striped with golden tint, but the *trage*-bearing chromosome having vanished, the feather will not be striped, but only bordered, and this border will be of a silvery, not of a golden tint.

Now we have succeeded in getting two samples of such cocks. From the crossing of the *trage-atuge* cock and *atrage-tuge* hen we reared two adult cocks. The prediction of the character of their exceptional feathers was quite justified. Thus golden unstriped feathers have not been found either in the hackles or in the saddle, but we have noticed the silvery unstriped feathers, quite similar to the feathers of true *atrage-tuge*, in great quantity (cf. Pl. II, fig. 6). The *atuge-trage* feathers were much rarer, but at some places—in the wings and tail—were found some feathers, one side of which was gold and barred and the other silvery but non-barred (cf. Pl. II, fig. 5).

This prediction being justified, confirms the supposition that the exceptional feathers are determined by the chromosome structure. This circumstance leads us to the following questions:

Haldane's (1921) investigations as well as ours (1922) had established that the genes *tuge* and *trage* show rather loose crossing-over, in such a way that their connection breaks off in approximately 30 per cent. This number probably varies with the age of the bird and with other circum-

stances, but of course it is easy enough to observe the breaking apart of these two genes. Now one ought to inquire what happens to the same genes by the somatic segregation (or by somatic non-disjunction). In other words, do the exceptional feathers always get both genes together, or does separation between them also occur?

The solution of this question is essential to the theory of the crossing-over. Morgan's point of view allows of crossing-over only at the moment of the “conjugation of chromosomes” in reduction division. On this interpretation the homologous chromosomes may exchange their parts and genes in no other cell division, and therefore in every cock's soma the sex-chromosomes ought to keep their initial composition, in the present $Tu Tg$ and $tu tg$ (F_1 , Plymouth Rocks \times Orloffs).

If crossing-over could also occur in somatic divisions or in the stage of the resting nucleus, there could be formed in the cocks' soma chromosomes of a new structure, namely *tuge-atrage* ($Tu tg$) and *atuge-trage* ($tu Tg$). Lack of the former of these two chromosomes would lead to the formation of golden-transversely-barred feathers; lack of the second would mean silvery-unbarred feathers.

The thorough examination of the feathers of the hybrids Plymouth Rock \times Orloff seems to confirm the possibility of such cases. It is true that it is not possible to determine the number of these crossings-over being 30 per cent. and so on. But in some cases we succeeded in finding some feathers, which, in the complete absence of transversely striped design, were distinctly silvery, *i.e.* *atrage-tuge*.

Two of these feathers are pictured on the coloured table (Pl. II, figs. 8, 9). It is true that inasmuch as these feathers are exceptional, the probability of chance errors is here very great. But in *atuge*-birds such silvery feathers are never found; there is no doubt about it. It is more difficult to guarantee the absence in this case of the *trage-phene*, *i.e.* the *manifestation* of *trage-gene*. The gene *trage* can manifest itself in different degrees. In *tifa* forms it is clearly manifested; but in *atifa* forms it is greatly weakened, especially in hens, where it is often very difficult to decide the question, whether she bears *trage* or not.

The feathers of the opposite type (*trage-atuge*) being so difficult to establish, have not been found by us. Therefore the problem of crossing-over in this case remains open. We can only state, that by “somatic segregation” the frequency of crossing-over is small, but we cannot confirm the non-existence of it, because of the “suspicious” feathers.

Does the phenomenon of “exceptional” feathers represent a pheno-

menon concerning only the sex chromosome? We can assert, that genes localised in the autosomes are able to give rise to similar exceptions in their distribution among feathers. We noticed that exceptional feathers are to be found among thoroughbred Plymouth Rocks, though almost always in hens. This fact is easily explained if one remembers that with fowls the female sex is heterozygotus, and therefore hens have only one sex-chromosome in the cells of their body, whereas cocks have two. If the sex-chromosome with *tuge* and *trage* does not fall on some feather of a hen, this feather will be deprived of both these genes and this will be immediately revealed. But with cocks it is necessary in order that the same effect be obtained, that both sex-chromosomes fall out. But this can only occur much more rarely than the missing out of one sex-chromosome, and therefore "exceptional feathers" are to be found in cocks much less often than in hens. Heterozygous cocks, however, are in this respect equal to hens, and even surpass them, for one can find several of these exceptional feathers on each heterozygous cock. We can even venture to observe that the constitution of such cocks seems to promote a disposition towards such exceptional feathers. We will speak of it again further on.

Therefore we must look among heterozygotes for exceptional feathers of another type than *tuge* and *trage*. And actually we find with our Plymouth Rock \times Orloff cock (N 2344), with which we began our investigation, a series of other exceptional feathers. We found black feathers, red and albinotic ones. This cock was really heterozygous for the *tifa*-gene (which calls forth black colouring in the middle of the feather), and for the *tedu*-gene (for colour as opposed to albinism). The presence of purely black feathers is explained by the fact that the cock is heterozygous for the *trakla*-gene, which is to be found in Orloffs but not in Plymouth Rocks. However, we have not yet investigated sufficiently the *trakla*-gene, to be able to make final conclusions.

We could discover through an examination of the "exceptional" feathers in this cock those parts of his hereditary formula in which he was heterozygous.

Are the exceptional feathers a peculiarity of Plymouth Rocks and their hybrids? We must point out the disposition of all birds having the *trage*-gene to have exceptional feathers. Birds without breed which had never been interbred with Plymouth Rocks, are inclined to have exceptional feathers as soon as they reveal *trage*-marking.

The *trage*-gene, or some other element closely related to it, brings into

the mechanics of mitosis some kind of disorganisation. The exceptional feathers of the hybrids *tuge* and *trage*, which have the structure $\frac{Tu\ tg}{tu\ Tg}$, are more often deprived of the *trage*-gene than of the *tuge*-gene (or at least of the phenes).

However, we can observe the same occurrence in the case of other genes, first of all in the case of *todi*-gene, which calls forth the epistatic white colouring. The *todi*-heterozygotes have among white feathers a certain quantity of pure black ones, or of some other colouring (blue, etc.), hens more often than cocks. They are very intensely coloured in distinction from some feathers of the albinotics of a light grey or yellow colour, and their colouring is exactly that, which it would be were the *todi*-gene to be removed. We have here a perfect illusion of the *todi*-gene having never been for some reason in these feathers. We can also observe that in the presence of the *tifa*-gene in the geno-type of the bird the quantity of exceptional feathers is greatly increased.

We can observe the same occurrence with the presence of the *tode*-gene. *Tode* inhibits the black pigment in the heterozygous condition until it becomes blue, and in the homozygous condition, until it becomes white. The blue colouring is to be found in Andalusian hens (with the presence of *tifa*), and among the blue feathers we can always find parts of feathers and even whole feathers of an intense black colour. These feathers seem to be deprived of the *tode*-gene.

The same occurrence is to be observed with the *tode*-homozygotes, and it is here even more striking. The exceptional feathers are not black among blue ones, but blue among dirty-white ones. As the dirty-white feathers have two *tode*-genes it is evident that the exceptional blue feathers are obtained by the falling out of one of the *tode*-genes. But while the falling out of the only *tode*-gene gives the heterozygotes at once a black colouring, here the falling out of one of two *tode*-genes gives a blue colouring.

We can sum up as follows: a series of colouring symptoms in fowls acts as if some feathers had not the genes which caused the given colouring. In the case of two genes localised in one chromosome (sex) they reveal a linkage or a repulsion according to the genetical construction, only with the difference, as compared with the gametes, that crossing-over cannot be either proved, or denied. But even if it exists, it exists in a much lower degree than in gametogenesis.

The above facts would have but a limited importance were we speaking of casual occurrences. But we already know of accidental elimination

of chromosomes in certain regions of the body for *Drosophila* in connection with the sex-chromosome in cases of gynandromorphism, etc.; and comparable phenomena have been found by Emerson (1921) in maize. Of these we can offer an explanation because the corresponding chromosome had at the same time two genes, one for colouring and the other for the structure of starch.

We think that the most interesting problem arising out of these facts is the problem of the localised piebald coloration. Considering different cases of piebald coloration in different animals we are able to make out a series of transitions from casual piebald coloration to cases where the piebald colouring is often distributed with great precision of localisation. The piebald coloration of rabbits and Jaroslaff cattle shows us a piebaldness that repeats itself in one individual after another with comparatively few variations. If the piebald colorations arise in connection with irregularities in the distribution of genes (or whole chromosomes) we are obliged to admit in the case of localised piebaldness the existence of a special mechanism that directs these irregularities. The admission of that kind of mechanism must revive the Roux-Weismann mosaic theory, and therefore the experimental verification of this theory would be very interesting and important.

Examining fowls we find some occurrences that are very interesting from that point of view. We speak of the development of red feathers on the shoulders and on the back of cocks. These feathers by their character and the original distribution of black and red pigments remind us very much of our exceptional feathers. They are very often "segregated" in two halves, one red, the other black, and very often there occurs a sharp transition from black feathers to red ones. We were not able to find any difference in principle between the colouring of the feathers of the back and the exceptional feathers. All the difference that is to be found consists in the fact that the appearance of these feathers in cocks is quite regular, and the place where they appear is exactly determined. Whether we have to do here with regularly appearing exceptional feathers, which appear owing to a special disposition on the part of cocks to give such exceptions, or whether it is merely an analogy, must be decided by further observations, and will probably require special methods of experiment.

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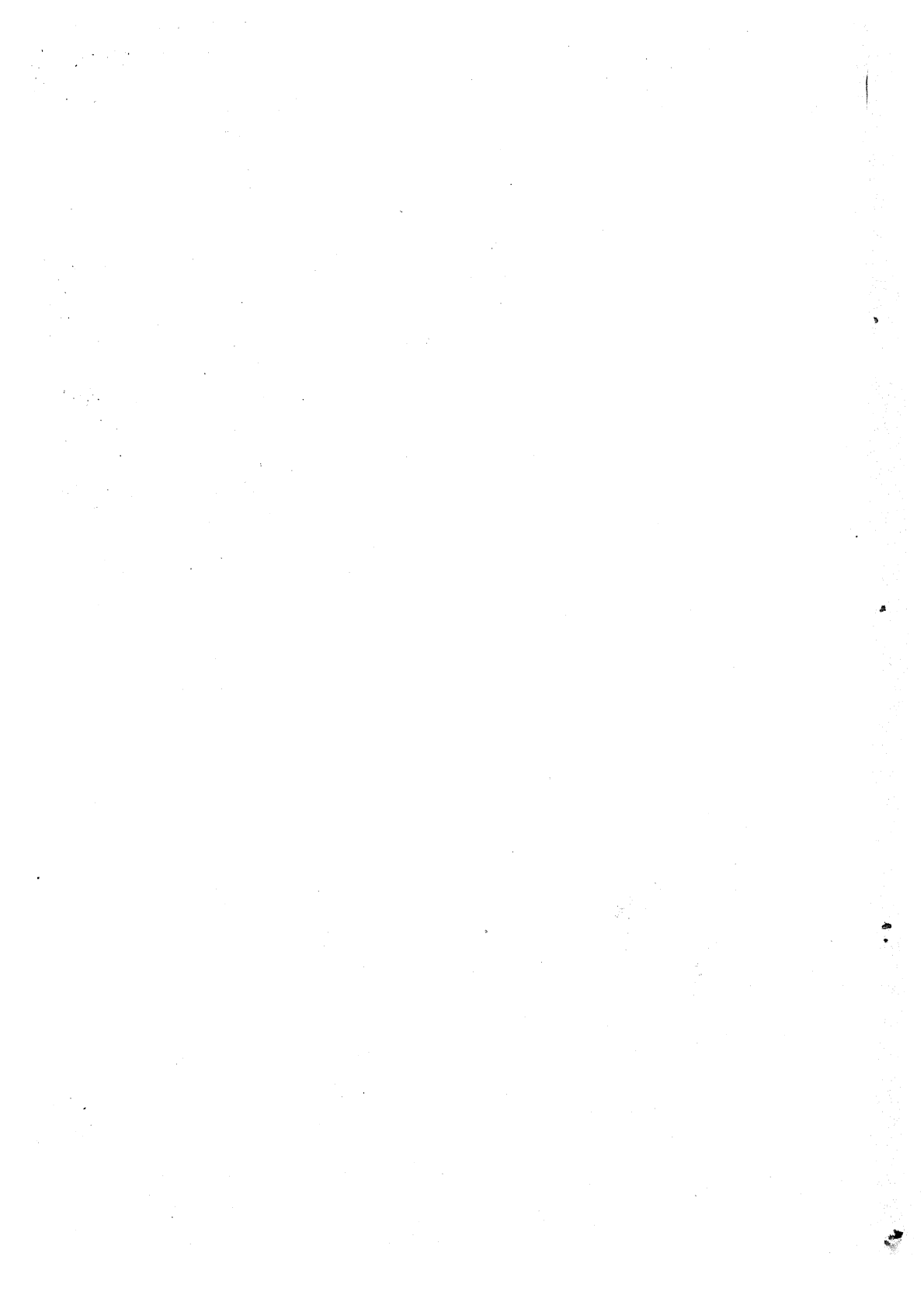
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EXPLANATION OF PLATE II.

- Fig. 1. Normal feather from cock N 2344 with the constitution $\frac{tuge\ trage}{atuge\ atrage}, \frac{tifa}{atifa}$.
- Fig. 2. Exceptional feathers from the same cock with "somatic segregation" in *tuge-trage* parts and *atuge-atrage* parts.
- Fig. 3. Normal feather from cock N 5334 with the constitution $\frac{tuge\ trage}{atuge\ atrage}, \frac{atifa}{atifa}$.
- Fig. 4. Exceptional feathers from the same cock with "somatic segregation" in *tuge-trage* parts and *atuge-atrage* parts.
- Fig. 5. Exceptional feather from cock N 6942 with the constitution $\frac{tuge\ atrage}{atuge\ trage}, \frac{atifa}{atifa}$, showing "somatic repulsion." Compare with the right feather of Fig. 2. The normal feathers from this cock are indistinguishable from Fig. 3.
- Fig. 6. Exceptional feather from the same cock, with *tuge* and without *trage*, as distinguished from Figs. 2 and 4, and according with the genetic constitution $\frac{tuge\ atrage}{atuge\ trage}$.
- Fig. 7. Exceptional very rare feather from cock N 2344 (see Figs. 1 and 2), probably without *tifa* as also without *tuge* and *trage* in most parts.
- Figs. 8 and 9. The rare exceptional feathers from the same cock, N 2344. Possible "somatic cross-overs" with *tuge* but without *trage* in most parts. Compare with Figs. 7 and 2.





STUDIES IN VARIEGATION. II. *HYDRANGEA* AND *PELARGONIUM*: WITH NOTES ON CERTAIN CHIMERICAL ARRANGEMENTS WHICH IN- VOLVE STERILITY.

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(With Five Plates.)

SINCE the foundation of this Institution in 1910, the genetic behaviour of a great number of variegated plants has been under observation, conducted by several of our staff. In general, our experiences have agreed with those of others who have engaged in similar work. Some of our types are, however, unfamiliar, or have exhibited features of special interest. In 1922 I undertook the breeding experiments which are here described. This led me to examine the anatomical relations of the parts, especially in *Pelargonium*, with the results now reported. Subsequently Mr Bateson asked me to prepare a general account of the work done here in so far as it relates to *Hydrangea* and Zonal *Pelargoniums*.

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HYDRANGEA.

Three types of variegation are represented in our series. Curiously enough, we have never seen a simple periclinal in *Hydrangea*. Of our white-over-green types, one has, additionally, green marginal lobes, and the other yellow lobes. As no seed was originally obtained from self fertilisation, two green types were introduced to serve as pollinisers.

As happens with many variegated plants, so especially in Hydrangeas, the several combinations of green, white and yellow, regarded as characteristic of the various types, show a high degree of instability. Not only do wholly green and wholly white shoots continually arise, but, even if the original combination persists, the distribution may change in detail. Only by the cutting out of undesired shoots can any one type be maintained.

Simple white-over-green periclinals would presumably, as in other white-over-green types, give only white seedlings.

Type A. Hydrangea hortensis var. *nivalis* (see Fig. 1) has the stems and the centres of the foliar organs solid white; the latter have a broad margin of solid green. This form, though frequently producing sports wholly green, shows otherwise great regularity of distribution in the type foliage.

This green marginate Type A ♀ × green (Type D) ♂ throws only green seedlings.

Attempts to use this form as a male have failed. Though little pollen is produced, it looks good, and few of the grains are distorted.

Type B. Hydrangea hortensis, variegated, with green lobes (see Fig. 2). This is a white-over-green periclinal, but differs from ordinary periclinal types in having lobes of solid green, without a white skin, irregularly distributed along the margin of the leaves. These lobes occur as out-growths from the leaf margin. They project from the general contour of the leaf, and their growth is more ample than that of corresponding parts covered by the white skin. Sometimes such lobes are few, but generally several are borne by each leaf. The marginal lobes are usually separated by a white interval from the general body of the green core, and, though seeming occasionally to touch the inner green, they are separate developments, distinct from the core.

The pollen shows a large proportion of good grains, but a few thick-walled grains are present. Such grains occur also in the A type and other Hydrangeas, and their significance is uncertain.

When a B type is fertilised by a green Type D male, it gives both white and green seedlings; when, however, it is used as a male on a green Type D female, only green seedlings result.

Type C. Hydrangea hortensis, variegated, with yellow lobes. This form is similar in chlorophyll distribution to Type B, but the lobes consist of pale yellow instead of green tissue. Here also the lobes are areas of rapid growth, and all degrees of lobing occur. The pollen is apparently all good.

When fertilised with pollen of a green (Type D), non-viable seedlings, some yellow and some white, result, whereas in the reciprocal cross, only green seedlings are produced.

Type D. Hydrangea Mariesii is a pure green form, showing no variegation. The pollen is very plentiful, but contains many spindle-shaped grains (which, unlike the others, do not swell in water), and a few thick-walled grains.

Type E. Hydrangea Mandschurica—a solid green, with a purple stem.

Emasculating such flowers is a tedious process and probably superfluous since the plants are in a high degree self-sterile. After this fact was ascertained our practice has been to choose inflorescences of the intended crosses, and to self-pollinate the numerous remaining inflorescences as a control. On two occasions only did these self-pollinated branches, although bearing vast numbers of flowers, produce any offspring, viz.

Type B in 1918 gave 8 seedlings, 6 green and 2 white.

Type D in 1923 gave 2 green seedlings which died in the cotyledon stage.

In 1922 emasculation was performed, but the ensuing results did not in that year differ from the rest. Evidently, therefore, even if occasional self-fertilisation did actually take place, no sensible disturbance was thus introduced into the numbers.

As already stated, the seedlings, regardless of the chlorophyll distribution in the male parent, *reproduce only the types composing the actual leaf margin of the female*. Thus, *nivalis*, having the margin entirely green, gives seedlings all green. Type B, white-over-green with white and green lobes, gives mixed seedlings wholly green or wholly white.

The actual appearance of the mother-plant may give an indication not only of the forms of the offspring, but of the numerical proportions in which they will be represented. Take, for example, the crosses of the yellow lobed form C with the green variety D as a male: in 1922, on the branch of C used, the proportion of yellow lobes was large, and the yellow seedlings were relatively many; but in 1923, when for want of a better, a branch with comparatively few and small yellow lobes had to be used, a much smaller proportion of yellow seedlings resulted.

Though fewer yellow seedlings are produced by a branch having few yellow lobes than by one having many, yet the frequency of yellow seedlings in the progeny of these types is greater than one would be led to expect from an estimate based on the appearance of the leaf and

leaf margin. In other words, relatively to the amount of the leaf margin covered by the lobes, they make a disproportionately large contribution to the offspring.

ZONAL PELARGONIUMS.

Among zonals variegated types exist in great variety. A few also are to be had in ivy-leaved and several in the scented-leaved sections. Curiously enough among the fancy or regal classes we have never seen or heard of a variegated form. As such plants would have been greatly esteemed sixty or seventy years ago when Pelargoniums were extensively bred, presumably they have not appeared.

In some varieties sports to all-green branches, or to all-white, or, in the case of periclinals, reversals, are common; in other varieties they are rare. Some notes on these manifestations are given in a subsequent section. Similar precautions to those already described in *Hydrangea* must be observed for the maintenance of the types.

In respect of chlorophyll development, three kinds of tissue occur, green, yellow and white, and of each of these there are minor modifications.

1. *Greens*. Doubtful variations in the depth of green occur, but we have not attempted to distinguish them.

2. *Yellows*. Of these there is considerable diversity. Probably with the possible exception of the lobes of Golden Brilliantissima, all the yellows are heterozygous combinations of green with a bleaching factor. The distinction between fuller or paler yellows is presumably an indication of a difference in the power of the bleaching factor. Some remain of the same tint throughout life, *e.g.* Zulu. Others become greener as the season advances, *e.g.* Marshal MacMahon and Harry Hieover; and a yellow which bleaches with age exists in the skin of Mrs Pollock and elsewhere.

3. *Whites*. The white tissue may be silvery white throughout its life; or in other forms it may be yellowish in the young state. The tissue bleaches to a clear white, but is liable, especially in older leaves and in winter, to become irrorated with green. A good example is Flower of Spring. This phenomenon, here called irroration, is probably similar to the assumption of a green colour by the old leaves in *Chlorophytum*¹.

On the leaf the irroration is usually at first most marked near the edges and veins, and a fully irrorated leaf looks as if dusted with a green powder. The change starts in autumn or winter, and is most noticeable

¹ Collins. *Journ. Gen.* Vol. XII. p. 1.

in early spring. Its incidence is always somewhat irregular, the middle leaves being commonly the most affected.

In the petiole a different state of affairs occurs. For instance in Mrs Mappin, a g.o.w.¹ leaved plant, irroration is confined to the central parenchyma which is enclosed together with the vascular bundles by a sclerenchymatous band. The irroration appears to be bound up in some way with the bundles, any unaffected areas being separated from the bundles by irrorated tissue. The sub-epidermal collenchyma also became greenish, otherwise none of the primary cortical tissue outside the sclerenchymatous band showed any irroration.

The two forms of whites are both incapable of separate existence.

Either yellow or white may co-exist in several periclinal combinations with green; only once have three kinds been found in combination, viz. in Golden Brilliantissima, which is a white-over-green, with yellow marginal lobes placed exactly as the green or the yellow lobes are in the *Hydrangea* types B and C. This yellow is quite distinct from that of the yellow *aurea* types. The common periclinals have one or other of the whites either as skin, or as core, in combination with green. Of periclinals combining green with one of the yellows, we have seen the full yellow, here called deep *aurea*, as a skin over green in several varieties, and the paler and much bleaching yellow as a skin over green in Mrs Pollock. This same pale yellow is recorded by Noack as having been raised as a substantive variety in his seedlings from Mrs Pollock.

Sectorial chimeras have been raised as seedlings, or sent in to us from time to time. Such irregular mixtures of tissues cannot be maintained permanently without great care, for when an orderly arrangement supervenes, either by the omission of one component, or by the combination of both in a periclinal system, the parts subsequently produced remains of that type unless a fresh disturbance occurs.

Several combinations have not occurred in our experience. We note especially that no yellow, whether of the deep or pale *aurea* has yet been seen by us in sectorial combination with either of the whites, but we suppose that this combination is among those with which Noack has been concerned².

The presence of detached marginal lobes was only seen in Golden Brilliantissima, where, as stated above, they are yellow. But in a variety recently received under the name of L'Yser, from Van Houtte, small

¹ G.o.w. is used here and subsequently to signify a green-over-white periclinal arrangement. W.o.g. has been used to signify the reverse, white-over-green, periclinal arrangement.

² Noack. *Verh. der Phys. Med. Ges. zu Würzburg*, N.F. Bd. 2, Heft 2, 1924, p. 72.

green areas occurred on the edge of a few leaves, which may or may not have been analogous.

Two very peculiar combinations, involving peripheral green, Freak of Nature and Happy Thought, have been much studied and attention should here be called to the fact that arrangements the converse of these have not been seen.

Freak of Nature (see Fig. 3).

This was introduced by Cannell in 1870. It is a very remarkable combination of green and white, having properties which at once distinguish it from any ordinary variegated plant. The green concerned is a full green, like that of normal zonals. The white is of the ordinary tingeing class, viz. though the white in fully grown organs is, at their maturity, a clear, bright white, yet white tissues in their young stage are yellowish; and as happens in other whites of this class, they are liable to become irrorated with green in later stages, especially in autumn and winter.

The stem, leaf-stalk and centres of all the foliar organs are white. The leaves have a broad peripheral band of green, which is usually much crumpled, and has the appearance of being too large for the leaf. The peripheral green tissue varies in width, commonly forming about two-thirds of the leaf. Exceptionally, especially at the apex of the leaf, the white reaches the margin dividing the green portion into two halves. *Flat*, free areas or segments occasionally appear in the marginal band. The margins of the stipules and sepals have a corresponding band of green, but there is no noticeable crumpling in them. In all the foliar parts the transition from green to white is abrupt. Usually the marginal green ends some little distance from the point where the leaf merges into the petiole.

The stems and petioles are usually white; but occasionally superficial and narrow green stripes can be traced running down them. Usually no such green band connects the nodes, and the internodes are wholly white. Certain peculiarities are noticeable in these green stripes which occasionally appear on the petiole. They may be dorsally placed (often appearing continuous with the green of one side of the leaf margin) in which event the cells constituting the green stripe are all thick walled and the sub-epidermis above this area is also fully green. Laterally placed green stripes are made up of normal parenchyma and the sub-epidermal layer above them is quite typical (*i.e.* not green).

Solid white branches are numerous. Often they contain no green,

apart from the irrorations to which any white tissue in this class of whites is liable; but not very infrequently, branches otherwise wholly white have minute ticks of green placed regularly on the margins of the stipules and more rarely on the limbs of the leaves.

Stems of the typical Freak of Nature flower freely. The flowers, however, are small, their stamens are aborted so far as to be scarcely visible in the perfect flower, and the carpels are very small, and continued upwards into stigmas of greatly reduced size. Apart from two exceptions (*v. infra*) these flowers produced no pollen and set no seed.

In contrast to these flowers, those borne on the sporting branches, whether green¹ or pure white, or green-over-white, have well-formed flowers, furnished with normal anthers, and pollen which looks good, and such flowers set seed without special difficulty. Experience, however, has been that flowers on the white stems do not set so readily as those on the green-over-white or green stems do. Nevertheless their ovaries and pollen look normal, and a fair number of seedlings have been raised from flowers on white stems.

The white branches occasionally have minute and sharply defined ticks of green in their stipules—a phenomenon quite distinct from the general irroration to which all of the white tissue is liable. From such a branch, two wholly green seedlings were once raised; presumably they traced their origin to such a—potentially—green area.

That the flowers belonging to the peculiar combination of green and white should be sterile, whereas the green and the white stems bear fertile flowers, constitutes a problem of considerable interest. The cytology has been examined by Mr W. C. F. Newton, who found that the development of pollen is normal up to the formation of the grains which separate from each other in the tetrads. After that, they perish for some unknown reason, and the anthers shrivel away. The megaspore mother cells up to diakinesis behaved normally, but they have not been traced further.

As exceptions, real or apparent, to the general sterility of these flowers, we have first an old record of six green seedlings raised from flowers on the typical tissue. They presumably came from a single truss, but no details were recorded. On a recent occasion, one flower in a truss formed a fruit, whether with a good seed or not is not known. The stem which bore it had a flat green sector on an associated leaf. We feel no serious doubt that in both these exceptions the fertility should be

¹ These green sports of Freak of Nature have the same plastid distribution and appearance as ordinary normal green Zonals.

regarded as contributed by a sporting sector, and not as evidence of fertility in the typical tissue.

Not many plants are known which have green and white parts combined as they are in Freak of Nature. In *Hydrangea* (var. *nivalis*) we have seen one possible example. Though in it fertility seems to be impaired, there was no actual sterility. In a variety of *Spiraea ulmaria* having the stems and central parts of the leaves white, sterility is complete on both male and female sides, the only example known to us which seems to be truly comparable¹.

Other plants having white stems and leaf-centres are under investigation here; for example, a well-known form of *Sedum Sieboldii* and a peculiar and unstable variety of Broad Bean (*Vicia faba*), but both are fertile, the first giving seedlings all green, and the second giving results too complex for summary description. Silver Kale also has a superficial resemblance, but is quite fertile, reproducing its special characters with some constancy. This last is evidently a combination of a different order, for we find it comes true from adventitious buds formed on its roots, whereas both Freak of Nature and the *Spiraea* give only albinos from their roots.

In connection with this problem, reference should be made to the departures from fertility seen in various periclinals mentioned elsewhere (p. 52).

Happy Thought.

Happy Thought is a much grown variety, combining green with a light and easily bleaching yellow tissue in a unique way, and having a peculiar and as yet unconformable genetical behaviour (Fig. 10).

The centres of the leaves are a pale greenish-yellow² and are surrounded by a broad green border. The pale greenish-yellow bleaches, becoming very pale yellow, and may subsequently be irrorated with green. The boundary between the two tissues is not quite sharply defined, and there is no folding at the leaf margin, as in Freak of Nature. The leaf surface is flat and, except for the chlorophyll distribution, similar in appearance to a normal leaf.

No sharp boundary can be observed between the yellow and the green margin when the leaf sections are examined. There is a region,

¹ See this *Journal*, xi. 1921, p. 96, Pl. XIV, fig. 2.

² Greenish-yellow has been used as a descriptive term here in the absence of positive evidence that the leaf centres are, or are not, normal *aurea* tissue. The colour, though not identical, approximates more closely to the skin of Mrs Pollock than to that of any other type.

several cells thick, where gradation from pale yellow to green occurs. The plastids of the pale yellow tissue are not easy to observe, but the cell contents present a yellowish appearance. The plastids are more obvious and greener in the area of gradation, and look quite normal and green in the green areas. Green plastids are sparsely distributed in the petiole.

The breeding results of Happy Thought have not been included in the general table, as the seedlings cannot be classified on any simple system.

Happy Thought selfed is recorded as having given:

- 26 greens,
- 19 old leaves green, young leaves yellowish,
- 16 „ „ yellowish, „ „ „
- 2 pale and non-viable,
- 1 variegated.

These results are not amenable to any simple classification and the experiment must be repeated.

Happy Thought stands in a class by itself despite some superficial similarity with Freak of Nature, for it shows no want of growth-correlation between its dissimilarly coloured tissues. Happy Thought is evidently not a periclinal, for the central *aurea* tissue of the leaves is not covered by any other. Nevertheless the geometrical arrangement of its parts is orderly, as probably also will its genetical behaviour prove to be.

The central tissue which is pale yellow is, when it emerges as a separate shoot, non-viable on its own roots. Presumably this yellow tissue is due to heterozygosis with one or more bleaching factors, the recombinations of which with each other and with the normal green are the source of the difficulty in interpreting the results of selfing.

Golden Brilliantissima.

This shows the normal general chlorophyll distribution of a white-over-green periclinal, but its variegation is still further complicated by the presence of lobes of yellow tissue distributed irregularly along the leaf margin. These lobes, which, as in *Hydrangea*, are regions of increased growth, are in young leaves of a yellowish green, become yellow, and finally, on the old leaves, bleach to nearly white.

Sporting appears to be infrequent in this type. Other than the occasional omission of the yellow lobes on the leaves, or the infrequent production of pure white leaves, only one sport has been observed. This was a yellow shoot on which the upper leaves and carpel walls were

yellow. The lower leaves when first noticed were white, but they may have been originally yellow.

Golden Brilliantissima sets seed badly; a large number of pollinations only produced 156 doubtful-looking seeds, of which 7 germinated. On the solid yellow sport just mentioned, however, three capsules gave 6 seeds, of which 5 germinated. Possibly this increase in fecundity may have been a consequence of the somatic simplification.

The seedlings of both sport and type were non-viable and did not get further in their development than the production of cotyledons. The cotyledons went through the same colour changes as the yellow lobes. None of the seedlings from the type plant were white, though whites were to be expected. In the similar *Hydrangeas* with marginal lobes, the offspring mostly agreed in character with the lobes; and here this disproportion was carried still further, no offspring at all reproducing the white areas on the margin.

Degrees of Sterility in various Combinations.

The peculiar somatic arrangement of Freak of Nature, as we have seen, involves complete sterility. Some of the orderly periclinals, especially the green-over-whites, are in general quite fertile, and several of the white-over-green set seed more or less easily. Wholly white branches from these forms set seed, but very sparingly, though their pollen used on greens is apparently fully effective.

Certain periclinals show striking abnormality of the reproductive parts. Madame Salleron¹, a well-known white-over-green, much grown as an edging plant, has never been known to flower, but when it reverses its tissues the green-over-white periclinal thus arising flowers freely, but is devoid of anthers. Its carpels and stigmas are small, and so far have never set in repeated attempts to fertilise them with various pollens. The presence of the white core is apparently not the cause determining this sterility, for the green-over-white has given us a wholly green branch, which, when propagated, has exactly similar defective flowers.

Why Madame Salleron is unable to flower we cannot say. Its leaves are quite flat, and their appearance does not suggest any incompatibility between core and skin. Moreover, *Manglesii*, a somewhat similar white-over-green periclinal, has plenty of flowers exactly like those of reversed Madame Salleron, and infertile as they are.

¹ H. Dauthenay (*Les Géraniums*, Paris, 1897, p. 164) states that the name should be as here given, and not Madame Salleron, the name in common use.

In connection with the sterility of these periclinals, mention must be made of three other periclinal types¹, in which, though no tissue deficient in chlorophyll is present, analogous sex-defects occur. Salmon Fringed, described in *Journ. Gen.* vol. XI. p. 91, has flowers wholly male, without female organs, whereas its core has fertile hermaphrodite flowers. Double New Life, a very peculiar form is, on the contrary, wholly female, devoid of male organs, but setting seed freely when fertilised with pollen of other types². As Dr Bond observed, "it gives out a normally flowered hermaphrodite sport, and we have also raised this sport from the roots of Double New Life, thus proving that the type is a periclinal combination, with a normally hermaphrodite core.

Kleiner Liebling, finally, is a plant of low restricted growth, with an abundance of small flowers, devoid of anthers, and having carpels and stigmas small and non-functional. From its roots we have raised a plant which has flowers quite normal and hermaphrodite, the form which doubtless constitutes the core of Kleiner Liebling.

It will be observed that in Salmon Fringed, the skin of a periclinal is male only, whereas in Double New Life, the skin is female only.

In Kleiner Liebling, the outer component is devoid of male organs, and its female organs are certainly defective.

All three, however, have normally hermaphrodite cores.

On present information, we have no clue to the nature of the sterility, which in these forms may be associated with the somatic combination of the various tissues.

In Freak of Nature we have the added complication that the sterility appears only in that special distribution of green and white peculiar to this type; for not only are its white and its green stems fertile, but also the periclinal green-over-white, in which evidently the same green and the same white co-exist³.

Seedlings.

The seedlings to be considered here are largely the result of crosses between the various sports of Freak of Nature. Others were made by crossing a g.o.w. plant here numbered $\frac{233}{20}$ with its green-flecked white sports.

The results agree fairly well with those obtained by Baur and Noack.

¹ Breeding experiments with these forms will be described subsequently.

² This was first discovered by Dr C. J. Bond, of Leicester, who raised plants from it and kindly supplied material to this Institution.

³ With all these we may compare the phenomenon seen in some of Winkler's graft-hybrids, in which the *combination* is sterile, though each component separately is fertile.

The high proportion of whites to greens in our families is noticeable. Noack obtained none and Baur only a small percentage. Here 23 whites occurred in a total of 78 plants. This distinction is probably due to the use of a slightly different class of white for breeding or may conceivably be an accident of germination.

The subsequent development of the seedlings was followed in detail in the hope of observing the development of some of the type forms. The development followed the lines originally described by Baur.

The salient features of families resulting from the various crosses may be summarised thus, the description being based on the appearance of the cotyledons: (1) green or g.o.w. branches selfed give only green seedlings; (2) white branches selfed give only white seedlings; (3) green or g.o.w. branches fertilised by pollen from a white branch give families which may consist of green, white and variegated seedlings; (4) white branches fertilised by greens or g.o.w. branches have so far given only 6 green and 3 variegated seedlings.

Except that seedlings with white cotyledons were non-viable, and that those with green cotyledons did not subsequently become variegated, the amount of variegation shown by the cotyledons was no sure guide to the subsequent appearance of the plant. For seedlings with strongly variegated cotyledons might show no variegation in the first leaves or subsequently, while those with only a trace of variegation in the cotyledons might develop into strongly variegated plants. In variegated seedlings the irregular and generally sectorial distributions of green and white characteristic of the first five, ten or more leaves is not maintained among their successors and the subsequent growth of the main bulk of the plants is green. In some the growing point became situated on white tissue in early life and the seedling died. A few seedlings developed an orderly periclinal system of variegation. The only other chimerical arrangement that is still represented is that in which the basal white shoots have appeared on plants otherwise green, or green or white basal shoots on periclinal plants. Periclinals¹ appear to be the most stable of the more usually produced types of variegation. Sectors and sectorials though common are more transitory in their nature, depending for their perpetuation on the disposition of the growing point. After a longer or shorter interval they develop solid uniform tissue or fall into a periclinal

¹ A peculiarity of w.o.g. leaved periclinals is that when planted out in summer, the leaves are upturned and buckled, but when brought into the houses in winter, their leaves are downturned and smooth. This difference is doubtless due to a change in differential growth rates of the two tissues. G.o.w. leaved plants show no such behaviour.

arrangement. The fact that whole branches, showing a different chlorophyll colour or a distribution different from that obtaining in the main shoot may be produced from the base has already been referred to. Such branches generally occur in the axils of old leaf scars¹ and may in seedlings be traced approximately to an area known to have produced tissue or tissues similar to that of the sporting branch in the leaves of earlier growth. Sectors can often be traced to an origin in the midst of dissimilar tissue and may there have had a spontaneous origin.

No lobed form, or anything resembling Freak of Nature, has yet appeared here as a seedling.

The fact that no periclinal has begun its seedling life *as such* is not easy to reconcile with Noack's view that periclinally leaved plants are due to a continual somatic segregation of the various tissues from the sub-epidermal meristem. We should anticipate that on this view regular distribution might occur as well in seedlings as in adult plants. Nevertheless no such seedling has arisen here, nor have we seen one reported elsewhere.

The development of graft hybrids² shows striking similarity with that of the variegated Pelargonium seedlings. The main growth stages shown by the one are to be found in the other. Here, too, are the early sectorial irregularities of distribution tending later to a more stabilised condition as uniform tissue or as periclinals.

Preliminary Notes on Anatomical Structure.

When we associate peculiar genetical properties with special periclinal arrangements, and no doubt rightly, refer these properties to those of a peripheral layer—the sub-epidermis, from which the germ cells are derived—it must be clearly understood that strictly speaking the special property of this layer is that which is visible in the lamina of the leaf and not necessarily elsewhere in the plant.

Strictly speaking the terms w.o.g. and g.o.w. are commonly misnomers except in application to the laminae of the leaves of most periclinals³. The two external layers of cells in stems and petioles are alike devoid of chorophyll, both in normal greens and in the periclinal

¹ The so-called sporting branches on the type plants often arise in the axils of old leaf scars or from the crown of the plant. With rare exceptions these branches consist of a rearrangement of tissues already existing in the plant, or they show solid uniformity of tissue by the omission of all but one of the component tissues.

² The graft hybrids referred to were made by Mr C. A. Jørgensen of Copenhagen, working at this Institution with Solanums, following Winkler's method.

³ For exceptions see later, pp. 57 and 58.

types. The layer below the epidermis has in all the stems and petioles a collenchymatous structure; and though undoubtedly in the w.o.g. types it must be genetically white, the only proof of any distinction obtainable by histological examination is the fact that by longitudinal sections this second layer of the stem can be traced into connexion with the white sub-epidermal layer of the laminae. Similarly though colourless in the stems this second layer both in normal greens and in g.o.w. is continuous with and traceable into the green sub-epidermal layer of the laminae. The curious fact that in g.o.w. *Pelargonium* (as also in g.o.w. *Coprosma*, *Vinca*, etc.) the stems are white¹ is of course a consequence of the absence of chlorophyll in the second layer of those parts.

The petioles of the two converse periclinals, though alike in having two colourless external layers, differ in the fact that w.o.g. has a median dorsal *white* stripe whereas in g.o.w. the corresponding band of tissue is *green*. The white stripe is of course continuous with the white leaf-margin, the green stripe similarly is continuous with the corresponding green margin².

The following account gives the chief features of the distribution of green plastids in the green types and in some of the definite periclinals. The distinctions between the plastids in the guard-cells in these several types are evasive and for the present a statement as to them is not attempted. In solid white branches no green plastids occur in stems, petioles or leaves.

Solid green plants.

A. *Stem*. Transverse section shows:

- (1) Colourless epidermis.
- (2) Colourless subepidermal collenchyma.
- (3) Several layers of primary cortex, all chlorophyll-bearing.
- (4) A more or less continuous vascular ring.
- (5) A central parenchymatous area in which chloroplasts are few.

The plastids in the stem are usually not so fully green and often appear smaller than those of the petiole or leaf.

¹ The g.o.w. *Euonymus* is a remarkable exception and in it the stem is apparently solid green and sections of it compared with those of a normal green show no very positive distinction. The anatomical relations of leaf and stem, however, are here very different from those in *Pelargonium*.

² Sometimes, particularly in g.o.w. leaved plants, the stripe is broken up into two or more stripes. Such irregularities can usually be traced into connection with irregularities in the distribution of tissues in the leaf. Sectors on the leaf can frequently be traced to a similarly characterised stripe down the petiole.

B. *Petiole* (see Fig. 5):

- (1) Epidermis colourless.
- (2) Colourless sub-epidermal collenchyma.
- (3) A fully green primary cortex.
- (4) A band of sclerenchyma completely enclosing the vascular system.
- (5) Widely separated vascular bundles.
- (6) Central parenchyma having fewer and paler chloroplasts.
- (7) A central and large vascular bundle.

C. *Leaf*. Sections show that except the vascular bundles little, if any, of the tissues contained within the sclerenchymatous band of the petiole enter the leaf. The primary cortex appears to be solely responsible for the tissues of the leaf blade. The sub-epidermis, unlike that of the petiole and stems, has thin walls and normal green plastids.

White-over-green Plants:

A. *Stems* as in the solid green plants.

B. *Petiole* (see Fig. 6) differing only from those of solid green plants in the possession of a dorsal median stripe of white, which in section shows as a plug of white tissue reaching down to the sclerenchyma.

C. *Leaf* has a white skin over a green core. This white portion at the margin becomes a broad solid white area. The leaf margins are continuous with the dorsal stripe on the petiole.

Green-over-white Plants:

A. *Stems* are solid white and show no green tissue in cross section.

B. *Petioles* (see Fig. 7), with the exception of a dorsal stripe of green, are solid white. This dorsal stripe, as in the w.o.g. plants, takes the form of a plug of tissue reaching the sclerenchyma; the sub-epidermal collenchyma above this region is colourless.

C. *Leaf* shows a distribution the converse of that described in the w.o.g. leaves above. Here also the leaf margins appear to be continuous with the dorsal stripe on the petiole.

Aurea-over-green Plants. The distribution is as in the w.o.g. plants, except that white tissue is replaced by one of the *aurea* tissues. As in the other plants the sub-epidermal collenchyma of the stem and petiole is colourless.

Special periclinal types:

Besides the typical periclinals already described, numerous rearrangements occur in various branches. For example, two distinct forms have arisen as branches on other plants, in which a really periclinal

arrangement was maintained throughout. The first was a branch on Freak of Nature. Externally the leaves and petioles were green and the stem was white, or greenish white. Anatomically the leaves were solid green and the white tissue was relegated in the petiole to the central parenchyma, and in the stem to the central parenchyma and the lower layers of the cortex (see Fig. 8).

The second was seen in two similar branches on a seedling $\frac{36^6}{22}$. This showed a distribution the converse of the branch on Freak of Nature described above. Green tissue appeared only in the central parenchyma and inner cortex of the stem (see Fig. 9), and in the central parenchyma of the petiole though occasionally invading the primary cortex, and in this event reaching into the base of the lamina of the leaf.

To such arrangements the term periclinal is strictly applicable. Similar distributions have been seen both by Baur and Noack, but they are, in our experience, rare.

The old theory that three layers are concerned in the formation of the leaf lamina is denied by Noack who asserts that, other than the epidermis, all the chlorophyll-bearing tissues of the leaf lamina are derived from the apical sub-epidermal meristem on the leaf. In variegated plants the chlorophyll character of the leaf is thus determined quite independently of that of the stem on which it is borne. In periclinals there is a latent labile gene present in the apical sub-epidermal meristem of the leaf which, during the transition of these cells from a "*meristematische*" to a "*halb-meristematische*" state, suffers a "*Determinations-prozess*" to white or green. In a w.o.g. periclinal, for instance, the apical sub-epidermal meristem would cut off white cells by radial, green cells by tangential walls.

To realise to the full the implications of this theory it is enough to state that the assumption of the independence of the chlorophyll character of leaf and stem is at variance with the existence of sectorials. That these sectorials, which bear white leaves on the white part of the stem, green leaves on the green part and variegated leaves on the borders of the two tissues, are merely a variant of the periclinal distribution is evidenced by the frequency with which these arrangements may be transformed one into the other. Further the theory requires that there should be a definite and regular proportion between the amounts of the tissues composing the core and of that composing the skin of the periclinal; instances of periclinals differing from each other in the proportion between the core and skin are given below.

In application to what I have called the typical or normal periclinals no one can deny that Noack's conception, though improbable, may be correct. But in any extended observation of variegated plants numerous redistributions and intermediate arrangements are continually seen, which seem impossible to represent or explain in terms of his scheme. Difficulty, for example, arises especially in regard to $\frac{36^6}{22}$ where, if present, the central w.o.g. area of the lamina is minute, and with regard to the more typical periclinal Golden Leaved in which the green core of the leaf is smaller than is usual and a nearly complete *aurea* band surrounds the petiole below the sub-epidermis.

Departures from regular arrangement continually occur simultaneously in a leaf and in the part of the stem associated with it, both in seedlings and in our original "type-plants." The simultaneity of these irregularities in leaf and stem preclude the supposition that the chlorophyll character may be determined by the apical sub-epidermal meristem of the leaf acting independently.

COMPARISON OF BREEDING RESULTS.

The various tissues and plants described in this paper obey very different laws of inheritance. The *aurea* tissue met with in *Pelargonium* provides the simpler cases, though it exists in three or more distinct forms. Two of these, e.g. Pale *aurea* and Deep *aurea*, are heterozygous combinations and differ perhaps only in the presence or absence of a bleaching factor. The third is represented here by the yellow lobes of Golden Brilliantissima. This plant has only given yellow non-viable seedlings when selfed. Until it has been crossed with a green no definite conclusions can be drawn as to the genetical constitution of the yellow lobes, but it seems possible that they may be comparable to non-viable homozygous yellows raised by Noack on selfing a Pale *aurea* form.

In *Hydrangea* the inheritance of the variously characterised chlorophyll tissues is strictly maternal. Variegated seedlings never occur.

The transmission of white tissue in *Pelargonium* is biparental but no definite ratios occur between the various types of offspring, and variegated seedlings are frequent.

It cannot be doubted that in both the case of *Hydrangea* and of the white tissue in *Pelargonium* the inheritance of the particular chlorophyll defect concerned is due to the transmission of some extra-nuclear material, probably plastids. Though the transmission of plastids by the pollen is still a vexed question yet the genetical evidence in

Pelargonium, for instance the occurrence of variegated seedlings, is strongly in favour of such transmission. In *Hydrangea*, on the contrary, the breeding results appear to prove that no such carrying over of plastids by the pollen occurs in these plants.

Hydrangea Breeding Results.

♀	♂	Family	Progeny																												
<i>Nivalis</i> . Type A	Solid green. Type D	$\frac{2}{18}$	About 200 all green.																												
<i>Nivalis</i> . Type A	Type D	$\frac{3}{18}$	12 green.																												
Green lobed white-over- green.		$\frac{1}{18}$	<table><tr><td></td><td colspan="6">March, 1918</td></tr><tr><td>Day</td><td>12</td><td>13</td><td>14</td><td>15</td><td>16</td><td>19</td></tr><tr><td>Greens</td><td>48</td><td>55</td><td>68</td><td>70</td><td>67</td><td>75</td></tr><tr><td>Whites</td><td>13</td><td>14</td><td>34</td><td>44</td><td>42</td><td>69</td></tr></table>		March, 1918						Day	12	13	14	15	16	19	Greens	48	55	68	70	67	75	Whites	13	14	34	44	42	69
	March, 1918																														
Day	12	13	14	15	16	19																									
Greens	48	55	68	70	67	75																									
Whites	13	14	34	44	42	69																									
Type B	Type D																														
Green lobed. Type B	Type D	$\frac{2}{23}$	Greens and whites as in $\frac{1}{18}$																												
Emasc.																															
Green. Type D	Green lobed. Type B	$\frac{1}{24}$	Many seedlings, all green.																												
Emasc. Type C	Green. Type D	$\frac{1}{23}$	4 whites seen (there may have been more); the rest of the family, consisting of many seedlings, was yellowish-green.																												
Yellow lobed white-over- green			<table><tr><td></td><td colspan="5">May, 1924</td></tr><tr><td>Day</td><td>1</td><td>3</td><td>6</td><td>7</td><td>13</td></tr><tr><td>Yellows</td><td>7</td><td>21</td><td>no count</td><td>24</td><td>2</td></tr><tr><td>Whites</td><td>—</td><td>20</td><td>33</td><td>42</td><td>9</td></tr></table>		May, 1924					Day	1	3	6	7	13	Yellows	7	21	no count	24	2	Whites	—	20	33	42	9				
	May, 1924																														
Day	1	3	6	7	13																										
Yellows	7	21	no count	24	2																										
Whites	—	20	33	42	9																										
Type C	Type D	$\frac{3}{24}$																													
Type D	Type C	$\frac{4}{24}$	Many seedlings, all green.																												
Type D	Selfed	$\frac{5}{24}$	2 greens germinated, but died without passing the cotyledon stage.																												

In 1922, families $\frac{1}{18}$ and $\frac{3}{23}$, the results as to viability were vitiated by the presence of woodlice in the seed pans. Special precautions were taken to obviate this cause of uncertainty in 1923.

In family $\frac{1}{18}$ the counts refer to the same pan of seedlings examined on successive dates. The numbers of whites observed on different dates must not be added together to show the relation of whites to greens, as, owing to the different times at which the whites die, many of the previous count are undoubtedly included in each figure given. On the other hand, as many have died between each count, the last number given cannot be taken as the total germination. Similarly, in $\frac{3}{23}$, where both yellows and whites are non-viable, the figures show only the relation on that date, though an effort was made, by lengthening the times between observations, to arrange counts so that all the previously germinated seedlings should have died. It cannot be pretended that this was accomplished. In both cases the numbers thus give only a rough indication of the proportions.

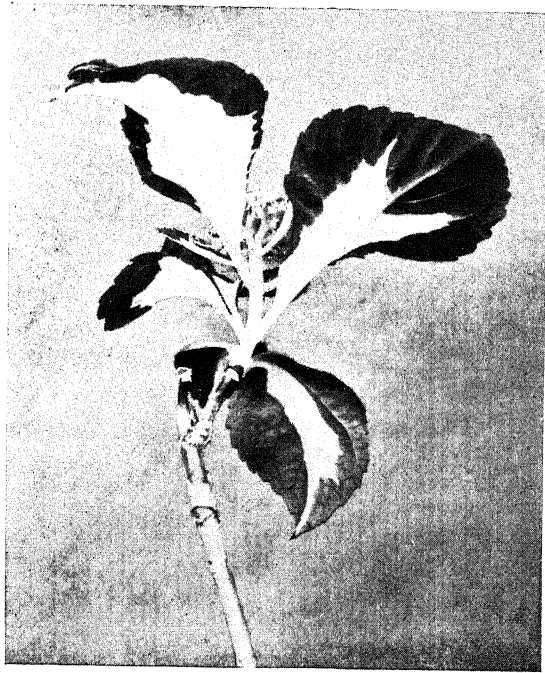


Fig. 1.





Fig. 3.

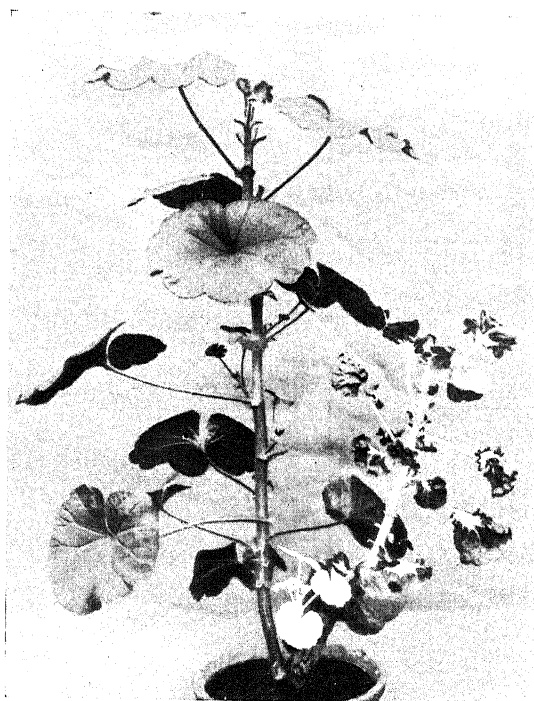


Fig. 4.

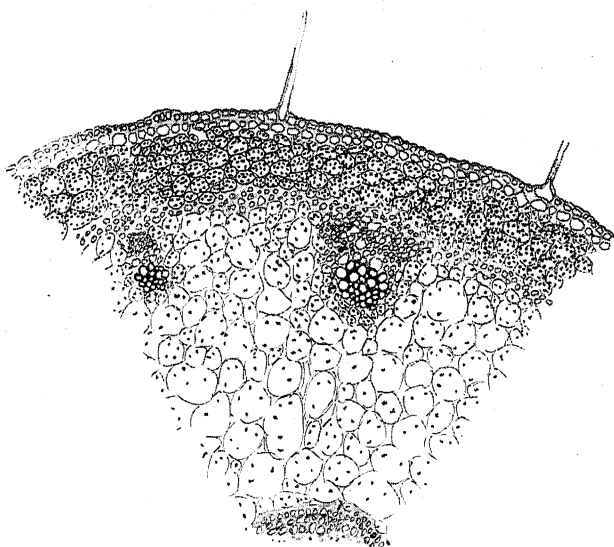


Fig. 5.

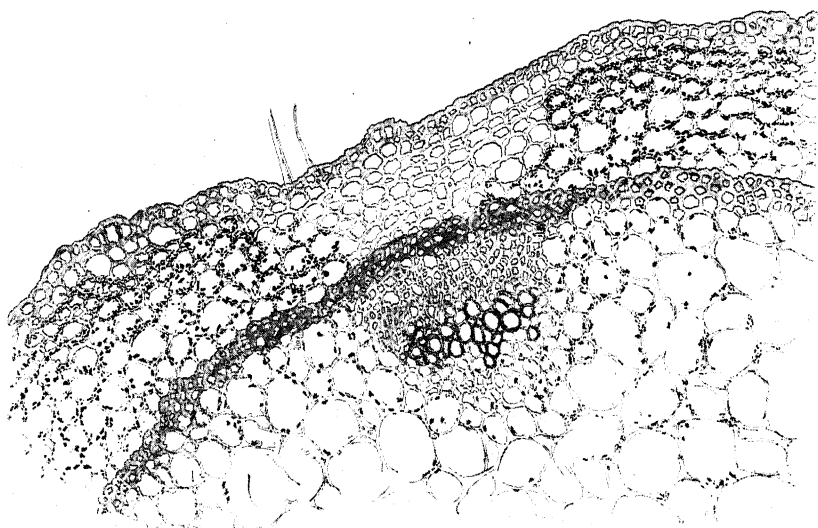


Fig. 6.

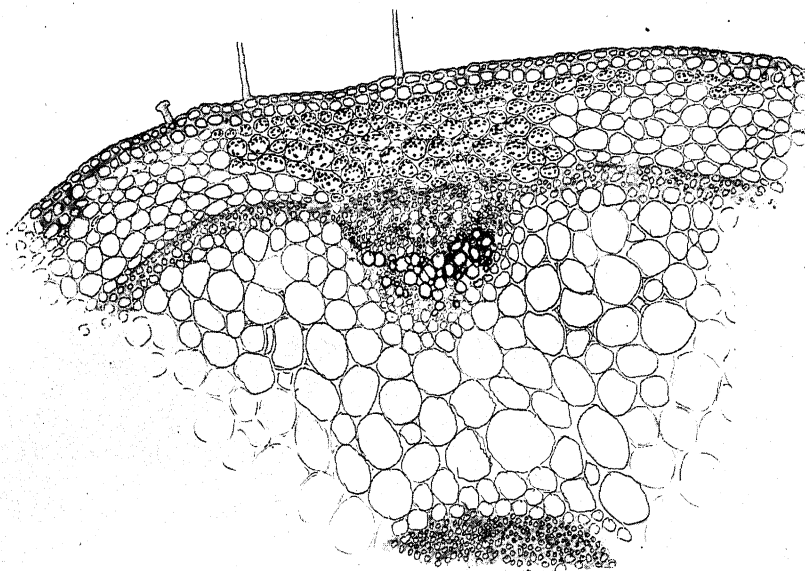


Fig. 7.

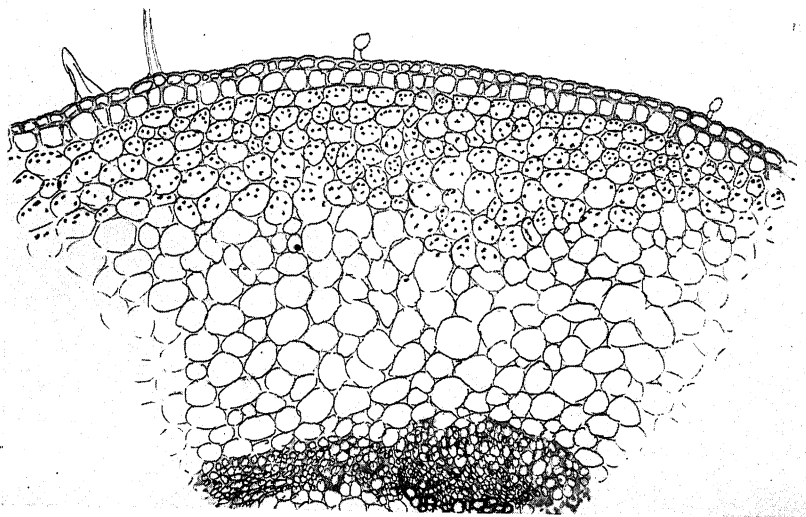
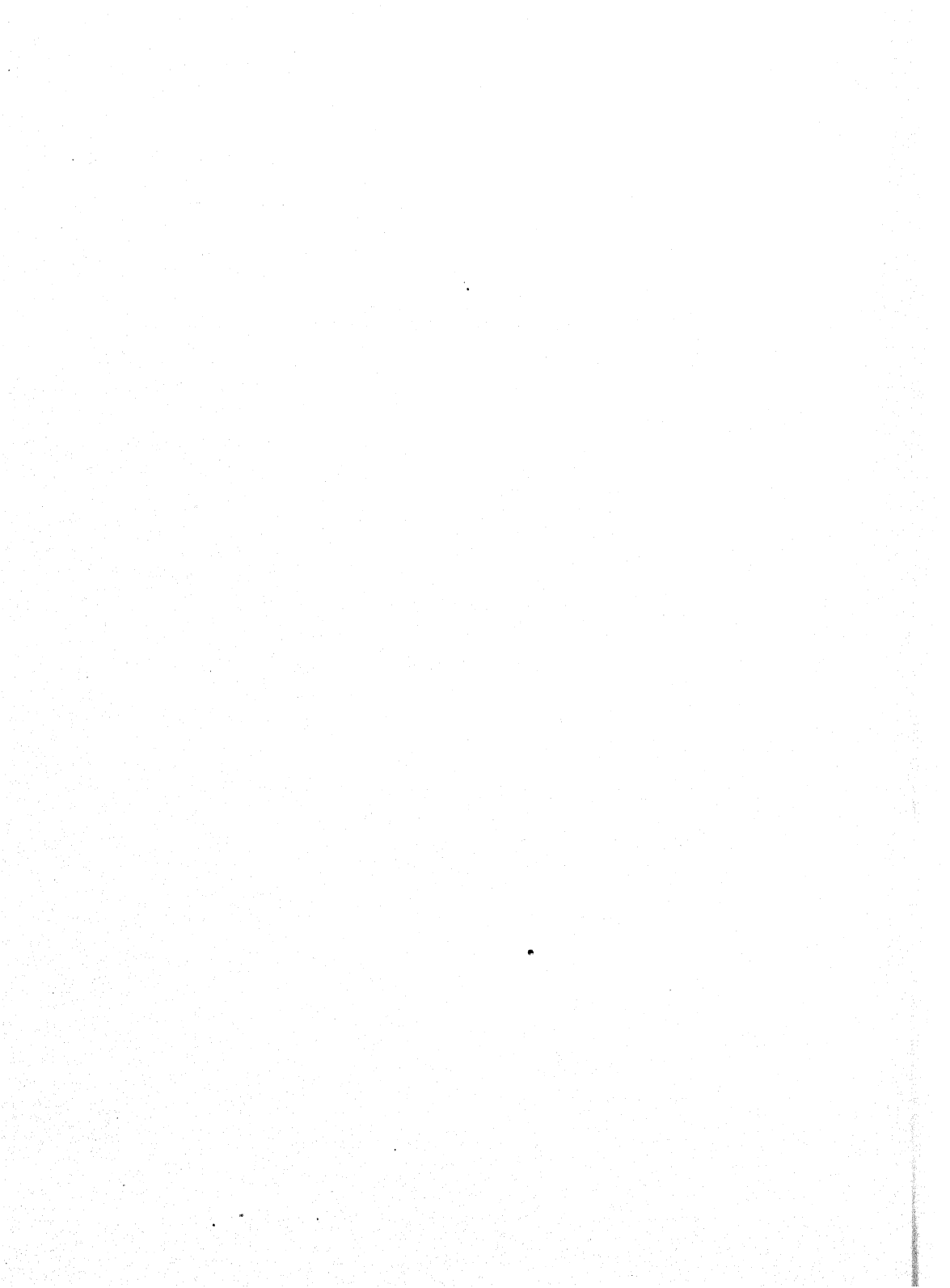


Fig. 8.



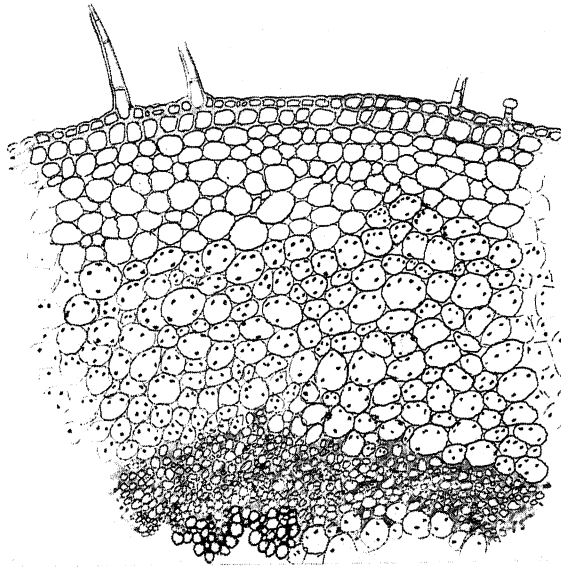


Fig. 9.



Pelargonium Breeding Results.

Results of self fertilisation of:

Solid green of g.o.w.

Freak of Nature, solid green	...	49 greens
" " " g.o.w.	...	8 "
$\frac{233}{20}$ g.o.w.	...	31 "
West Brighton Gem g.o.w.	...	54 "

Solid white and w.o.g.

Freak of Nature, white	...	17 whites
Snow Queen, w.o.g.	...	2 "

Results of crossing:

Green ♀ × g.o.w. ♂ and g.o.w. ♀ × green ♂.

Salmon Vesuvius (green) × West Brighton Gem (g.o.w.)	...	3 greens
Double New Life (green) × " " " "	...	4 "
West Brighton Gem (g.o.w.) × Salmon Vesuvius (green)	...	22 "

Green or g.o.w. ♀ × white ♂.

Freak of Nature (green) × Freak of Nature (white)	...	14	3	15
Salmon Fringed (green) × " " " "	...	3	5	13
Freak of Nature (g.o.w.) × " " " "	...	—	—	2
$\frac{233}{20}$ (g.o.w.) × $\frac{233}{20}$ white with green flecks	...	6	4	13

White ♀ × green or g.o.w. ♂.

Freak of Nature (white) × Freak of Nature (green)	...	—	1	—
$\frac{233}{20}$ white with green flecks × $\frac{233}{20}$ (g.o.w.)	...	—	2	6

DESCRIPTION OF PLATES III—VII.

- Fig. 1. *Hydrangea hortensis* variety *nivalis*.
- Fig. 2. *Hydrangea hortensis* variegated with green lobes.
- Fig. 3. *Pelargonium*, Freak of Nature, type plant.
- Fig. 4. *Pelargonium*, Freak of Nature, showing sporting branches.
- Fig. 5. *Pelargonium*, Mrs J. Smith—a solid green plant. A transverse section of the dorsal area of the petiole is figured showing the distribution of green plastids.
- Fig. 6. *Pelargonium*, Caroline Schmidt—a typical w.o.g. periclinal. The figure shows the distribution of plastids as seen in a transverse section of the dorsal area of the petiole.
- Fig. 7. *Pelargonium*, Baur's B—a typical g.o.w. periclinal. A transverse section of the dorsal area of the petiole is figured showing the distribution of green plastids.
- Fig. 8. *Pelargonium*, Freak of Nature—a g.o.w. stemmed branch with solid green leaves showing the distribution of plastids in the stem as seen in transverse section.
- Fig. 9. *Pelargonium* seedling $\frac{36^8}{22}$ —a w.o.g. stemmed branch with solid white leaves showing the plastid distribution in the stem as seen in transverse section.
- Fig. 10. *Right-hand plant*: typical Happy Thought. *Left-hand plant*: upper part typical. From the base arise a wholly green shoot, and a pale-yellowish shoot of the same colour on the interior of the leaves of the type.

ON A MONSTROUS FLOWER AND ITS LINKAGE IN THE JAPANESE MORNING GLORY¹.

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Komaba, Tōkyō.)

(With Four Text-figures and One Plate.)

INTRODUCTION.

THE Japanese morning glory (*Pharbitis Nil*) is very commonly cultivated in Japan as an ornamental flower; it contains exceedingly numerous forms. The attention of our cultivators has been called to two points, the size of the flowers and their abnormality. As to the latter, quite a number of monstrous types are found in our morning glory. In the present paper we shall describe the hereditary behaviour of one peculiar type of the flower, called by our gardeners "Shishi," and discuss its linkage with a leaf form.

Genetical studies on the abnormal flowers of this plant have already been undertaken by several investigators. According to Toyama (1916) and Imai (1920) the five-lobed flower acts as a simple recessive to the normal, funnel-shaped flower. This abnormal flower always accompanies the so-called maple leaf, which is usually five-lobed, the normal leaf being three-lobed. Two types of the so-called cup flower were studied by Miyake and Imai (1920) and Imai (1920, 1924 *b*); the one always accompanies a crapy leaf, while the other does not. Both cup flowers act as recessives to the normal. The papers by Imai (1920, 1924 *a*, 1924 *c*) and Hagiwara (1921) also dealt with other abnormal flowers. As to the doubling of this plant several forms were reported from the genetical point of view. According to Takezaki (1918) a form of doubling with peculiar leaves having a long petiole, called "Tenaga-Botan," is segregated from the heterozygous single flower by the Mendelian rule. Miyake and Imai (1920, 1921) showed two types of genetically different recessive doubles, both due to petalody; the one accompanies the "peacock" leaf, but the other does not. They also investigated a duplicated double

¹ The results of the study on coupling were published in the *Botanical Magazine*, Vol. xxxv. pp. 101-115, 1921, in Japanese.

with no accompanying traits (1921). This duplicated form is segregated as a recessive from the heterozygous single flower.

The double flowers of the Japanese morning glory may be divided into two groups artificially, the one is the double proper, containing several forms due either to petalody, petalomany, or duplication of flowers, and the other the "Shishi" flower, which will be discussed in the latter part of this paper. Two papers which dealt with the "Shishi" flower were published by Sô and Nishimura (1919) and Imai (1924 *b*). Our "Shishi" form having a "grasp" leaf seems to be the same as that studied by the former authors, and the factors concerned may also be common.

ORIGIN OF PEDIGREES.

From a packet of seeds furnished by a nursery firm we raised 15 plants all bearing the variegated leaves. According to the differences in the other traits they may be grouped in the following three classes:

Class I. Normal-formed and punched leaf, single flower (counted 4 individuals).

Class II. Normal leaf with roundish lobes, the surface of leaf punched and rolled up, single flower (counted 10 individuals).

Class III. Heart-shaped, and strongly rolled, forming the "grasp" leaf, flower blooms into "Shishi" (counted 1 individual) (Fig. 1).

These plants were bagged and their progeny traced, with the exception of the "Shishi" plant which gives no seeds. In raising the next generation we found that four plants of class I all bred true to the parental form, while ten plants of class II each formed a segregating family. The actual data of such segregating families are shown in Table I.

TABLE I.

Pedigree number	Normal			Roundish normal			Heart			Total
	Punched single	Rollled single	Grasped "Shishi"	Punched single	Rollled single	Grasped "Shishi"	Punched single	Rollled single	Grasped "Shishi"	
D 56	8	—	—	—	3	—	—	—	1	13
D 57	22	—	—	—	33	—	—	1	—	68
D 58	7	—	—	—	25	—	—	—	13	53
D 59	6	—	—	—	10	—	—	—	8	24
D 60	5	1	—	—	10	—	—	—	8	24
D 61	13	—	—	2	19	—	—	—	16	50
D 62	11	—	—	—	17	—	—	1	10	39
D 63	15	—	—	2	23	—	—	—	15	55
D 64	10	—	—	1	17	—	—	1	5	34
D 65	29	1	—	3	56	—	—	—	27	116
Total	126	2	0	8	213	0	0	3	124	476
Expected	116-150	2-833	0-017	2-833	232-334	2-833	0-017	2-833	116-150	476

A glance at the table shows that such an abnormal segregation might be due to the result of the linkage which takes place between the heart



Fig. 1. Portion of stem with grasped heart leaves ("Shishi").

leaf and the "Shishi." Before going on to examine this special relation it may be necessary to consider what sorts of allelomorphs are concerned in this cross.

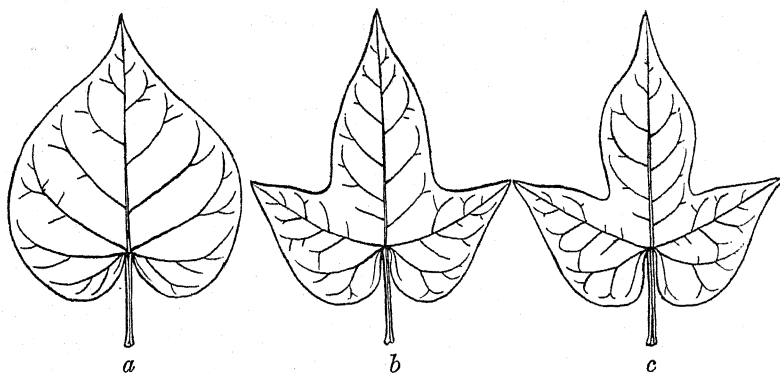
HEART LEAF.

As was stated before, the 15 original plants were composed of four normals, ten roundish-lobed normals and one heart leaf. With the exception of the last one, they all produced progeny; the first type each propagated a pure family, while the second one segregated into three phenotypes of normal (Fig. 2 *c*), roundish normal (Fig. 2 *b*) and heart (Fig. 2 *a*) as follows:

TABLE II.

	Normal	Roundish normal	Heart	Total
Observed	128	221	127	476
Expected	119	238	119	476

Thus the segregation occurred about in a 1 : 2 : 1 ratio, showing that the parental plants bearing roundish-normal leaves are heterozygous for

Fig. 2. (*a*) Heart, (*b*) Rounded, (*c*) Normal.

the leaf form. The hereditary relationship of the heart leaf to the normal has been studied out by several authors, of whom we cite Tanaka (1915), Sô and Nishimura (1919), Imai (1920) and Hagiwara (1921).

In raising a further generation we had the following facts: 91 plants bearing normal leaves gave pure families containing a total of 2449 individuals, while 164 roundish-normal leaves segregated into three forms of normal, roundish-normal and heart leaves. The summarisation of the data of the latter is given in the next table:

TABLE III.

	Normal	Roundish normal	Heart	Total
Observed	874	1849	642	3365
Expected	841.25	1682.50	841.25	3365

A marked deficiency is apparent in the heart leaf class, but this may be due to the mortality of the "Shishi" plants, of which the main part of the members of this class consisted. The record of the individuals of this generation was made when the plants had recovered their growth after being transplanted in the field. It is possible, therefore, that we may have failed to note certain plants which died early after transplantation, without their traits being recorded. On account of long-continued dry and hot days in this season, many weak seedlings were damaged, especially those of the "Shishi" strain, which were especially weak. As the observation of the first segregating generation was made just at the time of transplantation, such a discrepancy of ratio may not have been noted. Actually there was a fair accordance between observed numbers and those calculated from a 1 : 2 : 1 ratio, as was shown before. So that the deviation in question may be attributed readily to the result of the high death-rate among the "Shishi" strain. Those families giving no "Shishi" flowers, but all singles even among the heart leaves themselves, then, might be expected to segregate fairly into a 1 : 2 : 1 ratio, because there is no reason for the occurrence of such an unequal mortality. The actual segregation of eight such families went as follows:

TABLE IV.

	Normal	Roundish normal	Heart	Total
Observed	47	125	56	228
Expected	57	114	57	228

Thus the deficiency of the third class in this table did not appear, proving the above consideration. In the next raising of the heart leaves we obtained only three families giving 57 hearts, as a result of the fact that the majority of them are accompanied by the "Shishi" flower.

With these experimental results we can fairly conclude that the normal leaf is transmitted as an incomplete dominant character to the heart leaf, giving a 1 : 2 : 1 ratio in the segregation of the hybrids. The possibility, thus, of inferring the heterozygous genotype from the phenotype will be of great convenience in our linkage study, as may be seen in the later pages.

THE "SHISHI" FORM.

Four original plants bearing punched leaves and single flowers each gave uniform offspring, while the other ten single-flowered plants with rolled leaves segregated into three classes as represented in the next table:

TABLE V.

	Punched single	Rolled single	Grasped "Shishi"	Total
Observed	134	218	124	476
Expected	119	238	119	476

The observed number appeared clearly in a 1 : 2 : 1 ratio, showing a monohybrid segregation. Thus the plants of the first and the third classes respectively may be expected to breed true to the parental type, while those of the middle class will again yield segregating families. The experimental test proved the case to our satisfaction with the exception of the "Shishi" flowers, which could not be examined on account of their sterility. The normal flowers with punched leaves propagated invariably a uniform offspring; 97 plants gave 2662 as the total in the next generation. 161 plants of the middle class, however, all gave segregated families as shown in the following table:

TABLE VI.

	Punched single	Rolled single	Grasped "Shishi"	Total
Observed	847	1781	581	3209
Expected	802.25	1604.50	802.25	3209

The deviation was conspicuous, but as we have suggested in the former section, it may have been caused by the unequal mortality which occurred after the time the seedlings were transplanted in the field. The segregation would have occurred in the proportion of 1 : 2 : 1, had the record been taken earlier.

The "Shishi" flower thus behaves as a simple recessive to the normal, and it is always accompanied by the rolled leaf, by which we can conveniently, without failure, detect the strain even in the cotyledonous stage of the seedling.

A word may be added at this point as to the behaviour of the grasped leaves of the "Shishi" flower. The common type of the "Shishi" flowers carries the rolled leaf, but the rolling state is not so pronounced as to form the so-called grasped leaf. The question therefore naturally arises as to why in our present study we had in the "Shishi" plants all grasped leaves. According to Imai (1924 *d*) there are two types of the so-called punched leaves, in which the margin of the lamina is somewhat rolled up and the surface is uneven. From the fact that our homozygous normal flowers carried the punched leaves we may conclude that the grasped leaf is a characteristic produced by the combined effect of the factors of the "Shishi" flower and the punched leaf. With only our results as a basis, we cannot say that the grasped condition of the leaf is due to

the direct effect of the "Shishi" factor. Strictly speaking, the representation of the "Shishi" factor in the leaf condition is a moderate "rolledness" (Plate VIII, fig. 8 c).

COUPLING DATA.

Notwithstanding the fact that the segregating mode of each allelomorphic pair was quite simple we obtained, as was shown in Table I, rather complicated results, when we considered their segregations together. This complication is partly due to the incomplete dominance of each one of the allelomorphic characters of the two pairs, but it must be mainly attributed to the result of linkage. By the incomplete dominance of the characters the fate of each plant may be predicted without making further experiments, namely, a phenotype predicts a genotype. And, further, we can directly estimate the percentage of crossover from the data on self-propagation without making back-cross experiments. If we represent the factor for a heart leaf by **h** and that for the "Shishi" form by **si** the following hypothesis may be made. Because the segregation is a coupling we may consider that a given chromosome in the germ-cells of the original hybrid parents carries **h** and **si** factors, while its homologous one contains both dominant factors. The genetical varieties of germ-cells produced in such plants should be **hsi** and **HSi** of the non-crossover gametes, and **Hsi** and **hSi** of the crossover gametes. By combination of these four sorts of gamete we should expect the following result:

Origin	Genetical composition	Characters		
		Leaf form	Leaf surface	Flower type
Non-crossover g. × non-crossover g.	HHSiSi	Normal	Punched	Normal
	HhSiSi	Roundish n.	Rolled	Normal
	hhsisi	Heart	Grasped	"Shishi"
Non-crossover g. × crossover g.	HHSiSi	Normal	Rolled	Normal
	HhSiSi	Roundish n.	Punched	Normal
	hhSiSi	Heart	Rolled	Normal
	Hhsisi	Roundish n.	Grasped	"Shishi"
Crossover g. × crossover g.	HHSiSi	Normal	Grasped	"Shishi"
	HhSiSi	Roundish n.	Rolled	Normal
	hhSiSi	Heart	Punched	Normal

In the first segregating generation, as the following discussion will show, we do not find any individuals which might be expected to be produced by the union of two crossover gametes. The respective origin of the doubly heterozygous individuals, however, can be theoretically considered to be either a case of non-crossover gamete × non-crossover gamete or of crossover gamete × crossover gamete. But, as will be shown

later, the percentage of crossover is very low in this case, and so it is impossible to suppose that the **Hsi** + **hSi** individuals would be produced with such a limited number in the experiment; theoretically only one such individual is expected in about every 6700 double heterozygotes. Actually the **HhSisi** individuals, so far as our experiment went, all segregated into coupling data. If the plants are produced by the fertilisation of two crossover gametes, **Hsi** and **hSi**, they must result in repulsion segregations in their offspring. So the doubly heterozygous plants obtained in our experiments may practically all be regarded as produced by the union of two normal gametes, **HSi** and **hsi**. To continue the representation of the actual results, in our experiment the number of individuals produced by the union of non-crossover gamete and crossover gamete was 13 in all, while of those having the origin of non-crossover gamete \times non-crossover gamete there was a total of 463. For the production of the zygotes in these quantities there would be needed just double the number of gametes. The number of gametes thus concerned should be, then, theoretically 13 crossovers and 939 non-crossovers; consequently the gametic ratio is 72.23 : 1. From this, the value of crossover is calculated as 1.37 per cent.

By means of 259 plants obtained in the first segregating generation, with the exception of those of the family D 58, we traced the next generation to see to what extent the above expectation agreed with the further data. Of these, 89 plants bearing three-lobed punched leaves and single flowers may be considered as carrying the genetical composition of **HHSiSi**, and be expected to breed true to the parental characteristics. Actually 2432 individuals of the expected form were produced as the next progeny. The other 156 plants having the doubly heterozygous traits, however, all segregated into several phenotypes, as is represented in Table VII. These plants being doubly heterozygous, the mode of their

TABLE VII.

The data of the doubly heterozygous families in the second segregating generation.

	Normal			Roundish normal			Heart			
	Punched single	Rolled single	Grasped "Shishi"	Punched single	Rolled single	Grasped "Shishi"	Punched single	Rolled single	Grasped "Shishi"	Total
Total	806	19	0	18	1698	8	0	29	557	3135
Expected	764.978	18.658	0.114	18.658	1530.184	18.658	0.114	18.658	764.978	3135

segregation was complicated and particular, as was observed in the foregoing generation. With the total number as a basis we calculate 1.18 per cent. of crossover (non-crossover gamete : crossover gamete :: 83.57 : 1).

This figure approximates that of the foregoing generation, showing clearly that the results were the same. If an estimate is made on the grand total throughout the two generations the average value of crossover is 1.21 per cent. (gametic ratio is 81.87 : 1). As to the origin of these two forms of genotype they must be considered to be derived from two normal gametes. Let us next describe the offspring of the plants derived from the union of a crossover gamete and a non-crossover gamete. By the combination of a non-crossover gamete carrying **HSi** and a crossover gamete containing **Hsi**, three-lobed and rolled leaves may be produced which would be expected to segregate in the next generation with respect to the factor for the "Shishi" only. The results obtained by two such plants are:

TABLE VIII.

Pedigree number	Normal punched single	Normal rolled single	Normal grasped "Shishi"	Total
D 60-14	5	6	2	13
D 65-63	1	2	1	4
Total	6	8	3	17
Expected	4.25	8.50	4.25	17

Thus the segregation occurred in a monohybrid scheme of the 1 : 2 : 1 ratio. The "Shishi" plants with three-lobed and grasped leaves are a new form which had never appeared in the doubly heterozygous families throughout two generations, as the result of high linkage. Theoretically only one such form in 27,000 individuals would be expected in our hybrid segregation. The single-flowered plants bearing punched and roundish-lobed leaves being regarded as having the **HhSiSi** composition, may be expected to produce offspring of the three leaf forms in the proportion of 1 : 2 : 1. The actual segregation went as follows:

TABLE IX.

Pedigree number	Normal punched single	Roundish normal punched single	Heart punched single	Total
D 61-30	1	12	4	17
-31	2	10	5	17
D 63- 5	29	62	29	120
-22	4	13	5	22
D 64-19	1	2	1	4
D 65- 2	1	0	1	2
-56	2	10	4	16
-70	9	16	7	32
Total	49	125	56	230
Expected	57.5	115	57.5	230

The parental plants of these families might be synthesised by the union of a non-crossover gamete carrying **HSi** and a crossover gamete

having **hSi**. Single-flowered plants with three-lobed and rolled leaves, however, may be regarded as being produced by an **hsi**-carrying non-crossover gamete and an **hSi**-containing crossover gamete, and they will give the following generation, where the segregation occurs only for "Shishi" form. Actually three plants gave the expected results as follows:

TABLE X.

Pedigree number	Heart punched single	Heart rolled single	Heart grasped "Shishi"	Total
D 56-5	5	5	3	13
D 62-20	10	19	9	38
D 64-25	2	3	1	6
Total	17	27	13	57
Expected	14.25	28.50	14.25	57

The single flowers with heart and punched leaves, which appeared in about one-fourth of the above total number, are another type never obtained in our doubly heterozygous segregation throughout two generations, where it might be expected theoretically to have the same chance of yielding the "Shishi" flower with the normally formed leaf.

From the above breeding experiments it may be clearly stated that two factors, **H** and **Si**, represent a linkage segregation of 1.2 per cent. of crossover. Owing to the fact that the dominance in each allelomorphic pair is so incomplete that the heterozygotes can be separated phenotypically from their respective pure form, the value was calculated directly by the data of the self-propagated generation, without making a back-cross to the double recessive.

A linkage case between the "Shishi," with the grasped leaf, and the heart leaf reported by Sô and Nishimura (1919) seems to be the same one as ours, the only differing feature being that the segregation is either coupling or repulsion. On account of the repulsion state of segregation they had no double recessives in the segregating families. To calculate on the basis of our hypothesis, there might theoretically be only one double recessive in about every 27,000 individuals. And so, they¹ have concluded that the repulsion must be either complete or very high.

REPULSION DATA.

In the progeny propagated by a plant (D 324) of unknown origin we recorded the segregation that involves repulsion in the characters under discussion. The original plant had little rolled and roundish-normal

¹ They did not make note of the heterozygous forms separately from their respective pure normal forms.

leaves, but it bloomed quite normally. On selfing this plant we had the following results:

TABLE XI.

Pedigree number	Normal			Roundish normal			Heart			Total
	Even single	Weakly rolled single	Rolled "Shishi"	Even single	Weakly rolled single	Rolled "Shishi"	Even single	Weakly rolled single	Rolled "Shishi"	
D 324	0	1	19	0	45	1	25	0	0	91
Expected	0.003	0.495	22.252	0.495	44.510	0.495	22.252	0.495	0.003	91

Thus the segregation appeared in the repulsion state, giving no double recessives. The original plant, then, may be considered to have the **Hsi**-carrying and the **hSi**-carrying chromosomes. On sexual maturity it will produce two sorts of gamete, **Hsi** and **hSi**, by the normal procedure and the other two sorts of gamete, **HSi** and **hsi**, by the crossover mechanism. In the above table we count 89 plants which are considered to be produced by the union of two non-crossovers, and two plants which are expected to be synthesised by the result of non-crossover \times crossover, while no plants thought to be obtained by two crossovers appeared. On the basis of these data the frequency of crossover is estimated to be 1.10 per cent. (gametic ratio is 1 : 90). As this value is not far from that obtained in the coupling data we may be considered to have had the same results in both coupling and repulsion cases. The "Shishi" form invariably accompanies the moderately rolled leaves in this case, while in the former case the grasped leaf was the accompanying character. The difference of this plant is, as already stated, due to the effect of a factor (**u**) which has the influence of producing the punched appearance on the surface of the leaves.

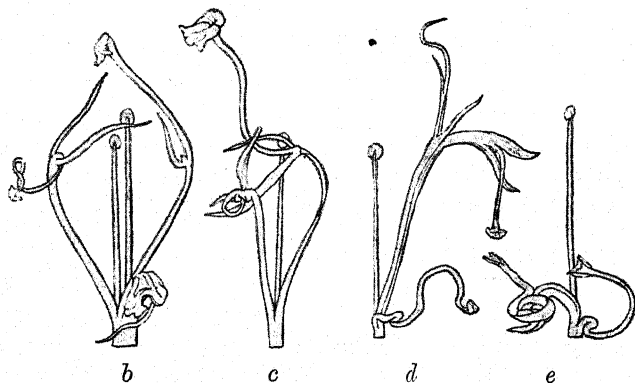
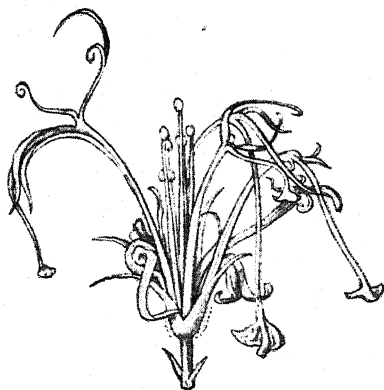
MANIFOLD EFFECTS OF THE **Si** FACTOR.

In the common double flowers the modification appears toward the inner part of the corolla, but in the "Shishi" strain some special malformations occur on the outside of the flower (Fig. 3). Though the sexual organs in the "Shishi" flower are present without marked degeneration morphologically, their function is much reduced. Consequently this strain usually produces no seeds. The flower has some fragmentary pieces on the outside of the corolla, thus forming a "feathering." The development of the feathers is varied in the different plants; this is also true even of the different flowers on the same plant. In the offspring of D 56-65 the variation in the "Shishi" flowers rarely went further in the direction of the extreme form in which the corolla was split into



Fig. 3. Two "Shishi" flowers.

a

Fig. 4. (a) An extreme form of "Shishi" flower in shape of a "crown."
(b-e) Dissected parts of the same.

five slender petals, and they again feathered and branched in shape of a "crown" flower (Fig. 4). On account of the sterility of the "Shishi" flowers, in practice they must be sought for among the offspring of the heterozygous normal plants. It is a well-known fact among our cultivators that the lineage of the "Shishi" may frequently depart from the ordinary culture in the course of raising further generations. The heterozygotes for the **Si** factor, however, can be picked out phenotypically by the weakly rolled state of leaves as was shown. If we keep this special feature in mind we need never lose the lineage of the "Shishi" flower. The rolled leaf and the monstrous flower are the manifold representations of a single "Shishi" factor. The rolling effect of this factor appears very early, even on the extended cotyledonous leaves of the seedling, the cotyledons being rolled up in a special manner.

The phenomena of manifold representations of a single factor are not rare in the Japanese Morning Glory; among them, the following examples may be cited. According to Takezaki (1918) the factor for a sort of double flower represents, beside the flower characteristic, also a long petioled leaf of a particular form. The **m** factor given by Imai (1920), and studied early by Toyama (1916), results in a split flower and the maple leaf. The **i** factor studied by the former author (1920) and Hagiwara (1921) is responsible for a "creased" flower and the "Rangiku" leaf. Imai (1920) further showed **d** and **t** factors which represent manifold characteristics respectively, the former for contraction of whole parts over the plant body, and the latter for the crapy leaf, cup flower and hairs on the outside of the corolla. His recent papers (1924 *a*, 1924 *c*) dealt with some other cases of similar phenomena in this plant.

SUMMARY.

1. The heart leaf (**h**) and the "Shishi" form (**si**) are respectively transmitted as recessives to the normal.
2. Owing to the incomplete dominance of the **H** and **Si** factors the segregating ratios are each a 1 : 2 : 1.
3. About 1.2 per cent. of crossover occurs in a linkage between these two factors. By the convenient nature of incomplete dominance calculation of linkage was directly made with the self-propagated specimens in the hybrid progeny.
4. The percentage of crossover is almost equal in the case both of coupling and repulsion.
5. The **Si** factor represents multiple effects on flower and leaf as well as cotyledon.

6. The grasped leaf found in the "Shishi" strain is due to the result of the additional effect of a punched factor (u).

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EXPLANATION OF PLATE VIII.

With the exception of Fig. 8, which was obtained from the progeny of D 324, all the figures in this plate were taken from specimens of the progeny of D 56-65.

Fig. 1. Punched normal.

Fig. 2. Punched roundish-normal.

Fig. 3. Rolled heart.

Fig. 4. Rolled normal.

Figs. 5 and 6. Rolled roundish-normals.

Fig. 7. Grasped roundish-normal ("Shishi").

Fig. 8. (a) even heart; (b) weakly rolled roundish-normal; (c) rolled normal ("Shishi").



a

b

c

GENETIC BEHAVIOUR OF THE WILLOW LEAF IN THE JAPANESE MORNING GLORY¹.

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(With Two Plates and Two Text-figures.)

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INTRODUCTION.

IN my previous papers² I have dealt with some leaf forms of the Japanese morning glory, *Pharbitis Nil*. The willow leaf, which I am now going to discuss, is another form found in this plant. The leaves are narrow and elongated just like those of the willow, so that the form is known as the willow leaf. The outside of the shoulder parts of the lamina are more or less protruded (Pl. IX, fig. 1), varying sometimes into real winged lobes, thus making a slender three-lobed leaf (Pl. IX, fig. 4). The flower of the willow leaf is invariably split into five narrow parts (Pl. IX, figs. 3 and 5); the type of the divided corolla is somewhat different from those of the other races, such as the maple and the "Sasa." The petals are more slender than those of the two above strains, being divided near the base of the flower tube. With the slender foliage and flowers the strain gives an impression of delicacy (Pl. IX, fig. 3). Introducing the double flower factor to this form our excellent cultivators produce one of the most admirable flowers, the flower being

¹ The substance of this paper was published in the *Botanical Magazine*, Tôkyô, vol. xxxviii, 1924, in Japanese.

² Imai, Y., "Genetic studies in Morning Glories. II, XI, XII and XIII," *Botanical Magazine*, Tôkyô, vols. xxxiv-xxxviii, 1920-1924, in Japanese.

divided into numerous narrow lobes to form a cluster of petals. The cotyledonous leaves of the willow are slender in form (Pl. IX, fig. 2), and the thigh of the two lobes is wider than that of the normal, these traits being more conspicuous in the "contracta" variety. The characteristics represented in such diverse parts of the plant body are transmitted together as a unit. Consequently the strain may be distinguished even in the seedling stage when the cotyledons are extended. Such a seedling selection is the method used widely in practice by our cultivators.

INHERITANCE OF THE WILLOW LEAF.

The willow form which was used widely as a maple parent in my breeding experiments bore the willow leaves, but the shoulders were not protruded like those above described. In the F_2 generation of the cross of this strain and the normal leaf there was dihybrid segregation of willow and heart leaves. From this experiment we are able to say that the willow strain now used is a willow carrying the heart leaf factor. So I may represent here the data obtained from the hybrids of unknown origin for the demonstration of the hereditary relationship between the willow and the normal leaves.

In 1919, I sowed a quantity of seeds bought from a seedsman, and reared six seedlings, all of which, with the exception of one which had the willow cotyledons, grew up with the normal leaves of the "contracta" variety. The exception was transplanted as a seedling into a pot, and allowed to grow there. The leaves of the seedling grew into the willow shape and it formed slender split flowers, but they produced no seeds. Thus my first willow plant died without giving progeny; luckily, however, some willow leaves reappeared in the offspring of the sister plants. Out of five normal plants, two gave a constant progeny of 52 and 32 normals respectively, whereas the remaining three threw out some willow leaves. The segregating numbers obtained are:

TABLE I.

Pedigree No.	Normal leaf	Willow leaf	Maple leaf	Total
Y 120	93	34	2	129
Y 122	38	9	0	47
Y 123	210	71(4)	0	281
Total	341	114(4)	2	457
Expected	342.75	114.25	0	457

The numeral in brackets denotes the number of the plants which made bud-variation.

Obviously the segregation occurred in a monohybrid fashion; the willow is thus a simple recessive to the normal. Quite unexpectedly,

however, I obtained two rogues which bore neither the normal nor the willow leaf, but the maple. How were these plants produced? Encouraged by the occurrence of such rogues I carefully re-examined the whole progeny in the field, and my labour was rewarded by the recognition of an abnormal individual. The plant was originally a willow, while later in the course of its growth it put out a branch bearing quite different leaves and flowers. I was able to identify this bud-sport as a maple. This occurrence of the maple branch on the willow plant seemed to give a clue to the cause of the appearance of the maple rogues. In the course of my experiment such abnormal occurrences, however, were found to be an habitual feature of the willow leaf. The sporting phenomena will be fully discussed in the later pages, so we may leave this subject for the present.

The examination of the next generation was made through the use of five normals, which gave the following results: One yielded 12 normal individuals, while the remaining four produced offspring comprising normals and willows in the proportion of three to one. The actual segregation was as follows:

TABLE II.

Pedigree No.	Normal leaf	Willow leaf	Maple leaf	Total
Y 120-1	8	2	0	10
-2	18	5	0	23
-3	12	3	0	15
-4	7	3	0	10
Total	45	13	0	58
Expected	43.5	14.5	0	58

From these, the willow-throwing families have been cultivated for many generations, but have yielded no new results beyond the expectation of Mendelian segregation and the rare appearance of the maple sports.

THE RELATION BETWEEN THE WILLOW AND HEART LEAVES.

With the pollen of a willow leaf (non-contracta) obtained from the segregating family of the heart leaf, a different line to that cited in the former section, I crossed with the normal leaf, and attempted systematically to trace the hereditary behaviour of the willow leaf in the hybrid progeny. As the willow leaf, however, is derived from the heart leaf it must be supposed to carry the heart leaf factor¹. The only visible

¹ Tanaka, C., *A text-book of genetics*, Tôkyô, 1915, in Japanese. So, M., and Nishimura, T., "Linkage in Morning Glory," *Journal of the Scientific Agricultural Society*, No. 208, 1919, in Japanese. Imai, Y., "Genetic studies in Morning Glories. II," *Botanical Magazine*, Tôkyô, vol. xxxiv, 1920, in Japanese. Miyake, K. and Imai, Y., "Genetic experiments with Morning Glories. III," *Ibid.* vol. xxxv, 1921, in Japanese. Hagiwara, T., "On the inheritance of linked genes in the leaf of the Japanese Morning Glory. II," *Journal of the Scientific Agricultural Society*, No. 224, 1921, in Japanese.

representation of the heart leaf factor on the willow leaf is the roundish shoulder of the lamina (Pl. IX, figs. 2, 3 and 5). Actually the parental willow, numbered as 505, bore willow leaves with roundish shoulders; consequently the winged lobes never develop in this willow strain. The F_1 plants obtained from the cross of the normal (65) and the willow (505) bore normal leaves, their main lobe being invariably roundish on account of the hybrids carrying the heart leaf factor in a heterozygous state. In the F_2 generation we obtained the following results:

TABLE III.

Pedigree No.	Normal leaf	Heart leaf	Willow leaf	Roundish willow leaf	Maple leaf	Heart-shaped maple leaf	Total
65 × 505-1	71	18	14	3	2	0	108
-2	31	10	10	2	0	0	53
-3	32	6	6	2	0	0	46
-4	58	15	14	5	1	0	93
-5	51	17	13	4	1	0	86
Total	243	66	57	16	4	0	386
Expected	217.12	72.38	72.38	24.12	0	0	386

In the above table the roundish normal leaves were counted together with the pure normals, since the identification of the former form might be accompanied by mistakes in this generation. As was stated above, segregation may be expected in both allelomorphic pairs, non-willow *v.* willow and non-heart *v.* heart, and this was actually the case. By free combination of the factors the willow leaf may be endowed either with the non-heart factor or with the heart one; those having the heart leaf factor should be the willow with roundish shoulders, while those carrying the non-heart leaf factor should be the so-called normal willow, the shoulders of the lamina being protruded. The willow leaves in the hybrid progeny cited in the former section invariably had protruded shoulders, showing that all specimens carry the non-heart leaf factor (**H**) in duplex, the segregation consequently occurring in a single pair of allelomorphs. The present segregation, however, should yield four forms, viz. normal, heart, willow and roundish willow leaves in the proportion of 9 : 3 : 3 : 1. Actually the observed numbers showed some deviation from the theoretical ones calculated on the basis of this dihybrid ratio. The comparatively low value of P ($= 0.11$) may be, however, due largely to the small number of specimens found in the willow class. Summing up, the non-willow is to the willow as 309 : 77, where we expected 289.5 and 96.5 respectively. The difference between the non-willow and willow is due to a single factor, so such a deviation from a 3 : 1 ratio cannot be considered to be the result of any disturbance in the factorial

relation. Consequently we can correct the actual data on the basis of a 3:1 ratio of non-willow to willow, and this may also be equally admitted in the segregation of non-heart and heart leaves. As the result of double treatment of correction the observed numbers will be changed into 216.72 normals, 72.825 hearts, 72.825 willows and 23.63 roundish willows, where we expected 217.12, 72.38, 72.38 and 24.12 respectively. Here $\chi^2 = 0.02$, consequently $P \approx 1$. The goodness of fit is very high, demonstrating free assortments of two factor-pairs. The data of the F_3 rearing are represented in Table IV. The results came quite according to expectation, i.e., normal leaves being pure for the **H** factor either bred true or segregated into normal and willow leaves in the ratio of 3:1 according to their genetic compositions, and the roundish normal leaves invariably segregated into either normal, roundish normal and heart leaves in the proportion of 1:2:1, or normal, roundish normal, heart, willow and roundish willow leaves in the ratio of 3:6:3:3:1, while the progeny of the heart leaves resulted in pure breeding and segregating. In the data of the offspring of the roundish normals, however, we had again some discrepancies in both segregating ratios, but these seem to be only a superficial deviation like that previously discussed. So the mathematical treatment on this point will not be repeated here. With the exception of the sporting phenomena, therefore, the segregating data obtained throughout two generations can easily be explained by assuming two factors, **h**, a heart leaf factor, and **w**, a willow leaf factor.

TABLE IV.

The F_3 data of the cross 65 \times 505.

Offspring of the normal leafed F_2 .

Pedigree No.	Normal leaf	Willow leaf	Maple leaf	Total
Total of 6 pedigrees	539	—	—	539
10	10	5	—	15
19	2	1	—	3
24	73	11	1	85
25	256	83	2	341
27	98	30	1	129
29	165	55	—	220
30	160	50	1	211
36	36	13	—	49
51	60	14	—	74
54	11	2	—	13
Total	871	264	5	1140
Expected	855	285	—	1140
47	48	—	15	63
Expected	47.25	—	15.75	63

TABLE IV (continued).

Offspring of the roundish normal leafed F₂.

Pedigree No.	Normal leaf	Roundish normal leaf	Heart leaf	Willow leaf	Roundish willow leaf	Maple leaf	Heart-shaped maple leaf	Total
5	2	4	2	—	—	—	—	8
18	33	99	36	—	—	—	—	168
28	5	10	3	—	—	—	—	18
41	5	37	6	—	—	—	—	58
42	11	16	12	—	—	—	—	39
43	4	8	1	—	—	—	—	13
46	17	55	20	—	—	—	—	92
Total	77	229	90	—	—	—	—	396
Expected	99	198	99	—	—	—	—	396
1	21	39	22	18	5	1	—	105
2	8	5	2	6	0	—	—	21
4	3	26	11	9	0	—	—	49
6	11	47	16	9 ⁽¹⁾	2	1	—	86
7	9	19	3	2	0	—	—	33
8	11	37	15	18	8	—	—	89
11	5	11	9	12	4	—	—	41
14	7	4	5	3	2	—	—	21
15	29	32	15	24	5	—	—	105
17	17	46	18	18	10	—	—	109
21	9	28	16	13	2	—	—	68
33	4	10	8	4	1	—	—	27
34	19	51	29	18 ⁽¹⁾	8	1	—	126
35	13	45	16	11	5	2	—	92
37	6	25	7	11	2	—	—	51
39	8	12	11	9	3	—	—	43
45	7	11	10	3	2	—	—	33
53	10	47	15	11	5	—	—	88
55	14	21	15	12	3	—	—	65
57	6	23	7	10	2	—	—	48
Total	217	538	250	221 ⁽²⁾	69	5	0	1300
Expected	243.75	487.50	243.75	243.75	81.25	—	—	1300

Offspring of the heart leafed F₂.

Pedigree No.	Heart leaf	Roundish willow leaf	Heart-shaped maple leaf	Total
Total of 4 pedigrees	270	—	—	270
3	21	2	—	23
12	90	19	—	109
13	49	18	—	67
20	101	39	1	141
38	28	4	—	32
40	43	19	—	62
44	93	21	—	114
48	26	6	—	32
56	96	32	1	129
58	58	13	—	71
Total	605	173	2	780
Expected	585	195	—	780

The numeral in brackets denotes the number of the plants which made bud-variation.

APPEARANCE OF THE FILAMENTOUS WILLOW LEAF.

From the seeds obtained of 326, a pure strain bearing the heart-shaped "Sasa" leaf¹, with the pollen of 505 I obtained F_2 plants bearing heart leaves. Both parents bloomed with split flowers, but not of quite the same type, the flower of the willow being split into lobes so slender that a tubular corolla might be obtained when five lobes are united edge to edge, while the lobes of the flower of the "Sasa" were much broader, though not so broad as those in the maple flower, and if we combine the edges of lobes the so-called "gentian" flower would be obtained. The heart leafed F_1 , however, bore normal, funnel-shaped flowers. The result thus shows us that both parental split flowers differ in their factorial pair, and working in a complementary way they produce the normal flower.

Speaking of the generation in F_2 (see Table V), there were produced besides the heart, the willow and the heart-shaped "Sasa" leaves, a few

TABLE V.

Pedigree No.	Heart leaf	Roundish willow leaf	Heart-shaped "Sasa" leaf	Filamentous willow leaf	Maple mutants	Total
326 × 505-1	35	8	6	2	—	51
-2	20	4	5	1	—	30
-3	37	12	9	1	—	59
-4	70	23	22	5	—	120
Total	162	47	42	9	0	260
Expected	146.25	48.75	48.75	16.25	—	260

leaves having the filamentous lamina (Pl. IX, fig. 6). This novel form may be called the filamentous willow leaf from its appearance in my *Pharbitis*-study. It blooms into quite a slender flower split into five filamentous lobes. We can therefore conclude that both parental plants carried the heart leaf factor, and so, we had no normal leaves in the descendants of the cross. Strictly speaking, therefore, the filamentous willow leaves which appeared in F_2 should be those of the heart leaf variety. As was stated, F_1 assumed the funnel-shaped flower, but this reversion is due to the recovery of two dominant factors for non-willow and non-"Sasa" in the hybrid. Consequently segregation of the flower type in the F_2 offspring occurred in a 9 : 7 ratio. In the above table the heart leaves all flowered normally, while the others bloomed with split flowers. Such complete correlations between the leaf form and the flower

¹ Imai, Y., "Genetic studies in Morning Glories. XIII. On the behavior of the 'Sasa' leaf and the phenomena of mutation in *Pharbitis Nil*," *Botanical Magazine*, Tôkyô, vol. XXXVIII, 1924, in Japanese.

type are due to the result of the manifold representations of each factor. The inheritance of the flower type can also be learned incidentally from the segregating data of the leaf form. We shall not attempt therefore to discuss again the subject of segregation of the flower type in the later generation.

The leaves of the parental 326 are of the heart-shaped "Sasa," but the margin of the lamina is much protruded. Such a protruded margin is not the subordinate characteristic of the heart-shaped "Sasa" leaf, but a modified form of it. I observed the entire-margined "Sasa" leaves

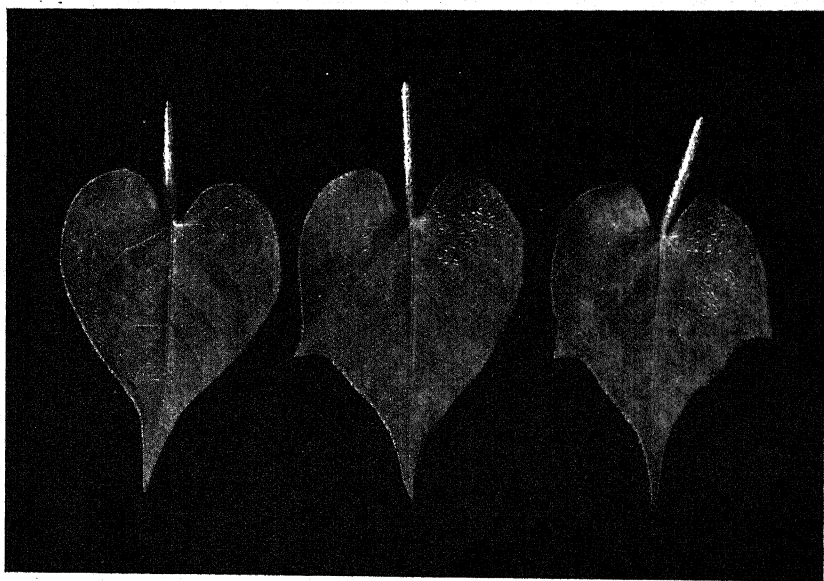


Fig. 1. One ordinary heart leaf and two protruded heart leaves.

in the other strains. And further, even in the present cross I met with the segregation of the entire and the protruded margins in the heart (Fig. 1), the heart-shaped "Sasa" and even in the roundish willow leaves, though the distinction between them cannot be clearly drawn. Such a fact may prove the above suggestion that the protruded margin is an additional characteristic of the heart-shaped leaves. And if I may be allowed a further suggestion I would say that the protruding margin may be due to the influence of a modifier or modifiers, and it seems to have a special relation to the "Sasa" leaf in segregation (linkage?). The extracted willow leaves in the F_2 generation were thus either entire-

margined or with protruding margin (Fig. 2). The latter may be mistaken for the normal willow leaves, if we do not know from which crosses they were derived. A large part of the filamentous leaves have the single lamina, but frequently they were mixed with some branched ones. All such protruding or branched laminae may serve to remind us of the modifying factor affecting the margin of the leaves. The hereditary behaviour of such a modifier will be one of the subjects of my future study. As was seen in Table V the segregating ratio of the four forms,

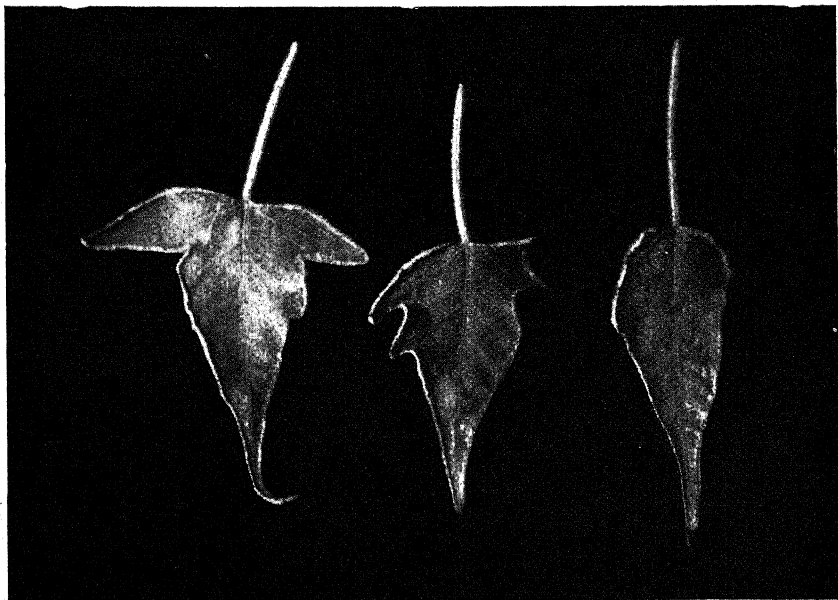


Fig. 2. Protruded poundish willow leaves.

heart, roundish willow, heart-shaped "Sasa," and filamentous willow leaves, was near 9 : 3 : 3 : 1. The factors segregated in this cross must be those of the willow and the "Sasa" leaves. So that the filamentous willow leaf is a double recessive form resulting from the combination of these two factors. On raising the F_3 generation, the examination was much limited on account of the sterility of some segregated forms; the willow and the filamentous leaves invariably produced no seeds, and many "Sasa" proved to be sterile. The actual results are shown in Table VI. With the exception of three "Sasa" families the table contains only the data of the progenies of double dominant F_2 . A glance at the

TABLE VI.

*The F₃ data of the cross 326 × 505.**Offspring of the heart leafed F₂.*

Pedigree No.	Heart leaf	Heart-shaped "Sasa" leaf	Roundish willow leaf	Fila-mentous willow leaf	Heart-shaped maple leaf	Heart-shaped maple "Sasa" leaf	Total
Total of 8 pedigrees	272	—	—	—	—	—	272
2	19	2	—	—	—	—	21
9	11	2	—	—	—	—	13
14	41	8 ⁽¹⁾	—	—	—	—	49
16	51	17	—	—	—	—	68
22	3	3	—	—	—	—	6
24	8	1	—	—	—	—	9
35	35	8	—	—	—	—	43
37	23	8 ⁽¹⁾	—	—	—	—	31
48	26	12	—	—	—	—	38
49	8	6 ⁽¹⁾	—	—	—	—	14
51	10	4	—	—	—	—	14
64	50	17	—	—	—	—	67
66	36	4	—	—	—	—	40
69	59	22 ⁽¹⁾	—	—	—	—	81
70	9	5 ⁽¹⁾	—	—	—	—	14
71	26	7	—	—	—	—	33
75	3	2	—	—	—	—	5
79	63	16	—	—	—	—	79
81	25	8	—	—	—	—	33
94	51	11	—	—	—	—	62
Total	557	163 ⁽⁵⁾	—	—	—	—	720
Expected	540	180	—	—	—	—	720
15	66	—	26	—	1	—	93
23	2	—	1	—	—	—	3
31	5	—	2	—	—	—	7
34	37	—	6	—	—	—	43
36	28	—	4	—	—	—	32
43	60	—	17	—	—	—	77
62	34	—	13	—	—	—	47
65	57	—	15	—	—	—	72
67	49	—	14	—	—	—	63
74	38	—	13	—	—	—	51
82	45	—	15	—	—	—	60
85	50	—	9	—	—	—	59
90	15	—	9	—	—	—	24
92	21	—	5	—	—	—	26
Total	507	—	149	—	1	—	657
Expected	492.75	—	164.25	—	—	—	657
1	40	4	7	1	—	—	52
3	21	7	4	1	—	—	33
4	13	4	1	0	—	—	18
5	37	2	8	0	—	—	47
6	9	3	4	1	—	—	17
8	4	1	1	1	—	—	7
10	43	11	10	1	—	—	65
11	24	9	6	4	—	—	43
12	27	12	9	2	—	—	50

The numeral in brackets denotes the number of the plants which made bud-variation.

TABLE VI (continued).

Pedigree No.	Heart leaf	Heart-shaped "Sasa" leaf	Roundish willow leaf	Fila-mentous willow leaf	Heart-shaped maple leaf	Heart-shaped maple "Sasa" leaf	Total
13	15	4	2	0	—	—	21
17	42	16	13	9	—	—	80
18	39	9	10	1	—	—	59
19	7	2	1	1	—	—	11
20	40	5	12	2	—	—	59
25	11	3	1	0	—	—	15
26	27	6	5	1	—	—	39
27	29	7	5	1	—	—	42
28	72	13	12	3 ⁽¹⁾	—	—	100
30	5	0	0	1	—	—	6
32	20	11 ⁽¹⁾	5	3	—	—	39
33	27	8	7	3	—	—	45
38	13	2	1	0	—	—	16
40	40	16 ⁽¹⁾	4	1	—	—	61
42	16	8 ⁽¹⁾	2	1	—	—	27
44	41	16 ⁽¹⁾	21	4	—	—	82
45	17	2	10	3	—	—	32
46	7	1	2	0	—	—	10
47	12	3	4	1	—	—	20
50	16	6 ⁽¹⁾	7	2	—	—	31
52	37	7	15	2	—	—	61
53	56	19	15	3	—	—	93
54	34	15	13	6	—	—	68
55	40	11	9	1	—	—	61
57	58	12	23	5	—	—	98
58	23	9	9	0	—	—	41
59	25	6	2	0	—	—	33
60	33	10	13	0	—	—	56
61	55	21	21	4	—	—	101
63	53	11	11	4	—	—	79
68	33	12	11	3	—	—	59
72	57	8	11	2	—	—	78
77	30	6	10	2	—	—	48
83	26	15	9	4	—	—	54
84	28	6	7	0	—	—	41
87	19	4	2	0	—	—	25
88	20	7	5	1	—	—	33
89	15	4	4	0	—	—	23
91	22	3	4	2	—	—	31
93	16	1	0	1	—	—	18
Total	1384	378 ⁽¹⁾	378	88 ⁽¹⁾	—	—	2228
Expected	1253.25	417.75	417.75	139.25	—	—	2228

Offspring of the heart-shaped "Sasa" F₂.

Pedigree No.	Heart-shaped "Sasa" leaf	Filamentous willow leaf	Heart-shaped maple "Sasa" leaf	Total
7	4	—	—	4
29	2	—	—	2
80	1	—	—	1
Total	7	—	—	7

The numeral in brackets denotes the number of the plants which made bud-variation.

table shows no new results beyond our expectation, so that no further discussion is necessary on the segregation of the factors in question. But a word will be added on the considerable discrepancy of the ratio observed in the result of the dihybrid segregation. Here the mathematical treatment shows that χ^2 is very high, numerically 37.91, so the value of P is almost zero. But this discrepancy in the ratio may be mainly due to the death of the recessive forms as a result of their weakness. If we correct the data on the assumption that the segregating ratio of each alternative character-pair must be 3 : 1, the numbers are 1243.08 hearts, 429.85 heart-shaped "Sasas," 429.285 roundish willows and 126.35 filamentous willows, where we expected 1253.25, 417.75, 417.75 and 139.25 respectively. Here χ^2 equal 1.91, so the goodness of fit is fairly high, numerically 0.59, showing about one chance of such an occurrence in every two trials.

THE PRODUCTION OF THE SPLIT WILLOW LEAF.

The type of leaves in a given strain is usually almost uniform in one individual, while it varies to a considerable extent in the leaves of the so-called "Rangiku," the sharp-pointed leaves irregularly being shaped and lobed. This strain blooms into a "creased" flower of funnel shape; the corolla consists of numerous petals and is frequently cut in one or two places. The cotyledons are shortened in the length of their lobes just like those of the radish, and sometimes are split in an irregular manner. These features are the multiple representations of a single "Rangiku" factor.

The experiments made by Hagiwara¹ and myself² showed that the characteristics are transmitted together as a recessive unit to the normal. The Japanese word "Rangiku" means "creased chrysanthemum," the name coming from its elegant flowering.

As the result of pollination with the pollen-grains of a willow leaf (505) on the stigma of a "Rangiku" flower (M 3) I obtained some F_1 plants bearing the roundish normal leaves and normal funnel-shaped flowers. These aspects as represented on the hybrid plants are entirely different from those of both parents, and the form is not that of any intermediate state. Such plants gave the F_2 offspring segregated as follows:

¹ Hagiwara, T., "On the inheritance of linked genes in the leaf of the Japanese Morning Glory. II," *Journal of the Scientific Agricultural Society*, No. 224, 1921, in Japanese.

² Imai, Y., "Genetic studies in Morning Glories. II," *Botanical Magazine*, Tôkyô, vol. xxxiv, 1920, in Japanese.

TABLE VII.

Pedigree number	Normal leaf	Roundish normal leaf	Heart leaf	"Rangiku" leaf	Willow leaf	Roundish Split willow leaf	Maple leaf	Total
M 3 × 505-1	3	4	9	9	6	1	0	33
-2	20		8	9	10	3	3	35
Total	27		17	18	16	4	3	86
Expected	36.28		12.09	16.13	12.09	4.03	5.38	86

TABLE VIII.

The F_3 data of the cross M 3 × 505.

Offspring of the normal leafed F_2 .

Pedigree No.	Normal leaf	"Rangiku" leaf	Willow leaf	Split willow leaf	Maple leaf	"Mitsuo" leaf	Total
6	40	—	—	—	—	—	40
4	38	9	—	—	—	—	47
9	18	2	—	—	—	—	20
10	60	21	—	—	—	—	81
19	9	3	—	—	—	—	12
Total	125	35	—	—	—	—	160
Expected	120	40	—	—	—	—	160
2	8	—	5	—	—	—	13
15	61	—	19	—	—	—	80
16	32	—	8	—	—	—	40
18	61	—	18	—	—	—	79
23	4	—	1	—	2	—	7
24	50	—	10	—	—	—	60
Total	216	—	61	—	2	—	279
Expected	209.25	—	69.75	—	—	—	279
1	105	33	33	8	2	—	181
5	7	2	4	0	—	—	13
11	61	28	20	1	—	—	110
13	52	12	18	9	—	—	91
17	25	5	6	3	—	—	39
Total	250	80	81	21	2	0	434
Expected	244.125	81.375	81.375	27.125	—	—	434

Offspring of the "Rangiku" leafed F_2 .

Pedigree No.	"Rangiku" leaf	Split "Rangiku" leaf	"Mitsuo" leaf	Total
Total of 3 pedigrees	17	—	—	17
3	30	7	—	37
8	14	2	—	16
12	9	1	—	10
20	9	1	—	10
22	0	1	—	1
Total	62	12	0	74
Expected	55.5	18.5	—	74

The forms produced were normal, roundish normal, heart, "Rangiku," willow, roundish willow and split willow leaves (Pl. X, fig. 7); among them, the last form was a novelty to me. The split willow leaf as it might be called, is a variety of the willow, but the margin of the lamina is serrated and sometimes split into two or three lobes. The flower of this strain is divided and creased, or in other words it represents both characteristics of the willow and the "Rangiku" flowers. The cotyledons are small in size and irregularly formed in various shapes and sizes (Pl. X, fig. 7), the extreme variation going to form tiny spatula (Pl. X, fig. 8). Even in one seedling both cotyledons frequently differ conspicuously in size as well as in shape.

On account of sterility of some F_2 forms the F_3 raising was successful only in the normal, the heart and the "Rangiku" leaves. After transplantation in the field, the majority of the F_3 seedlings were, however, damaged by continuous rain, so the records made after that time do not contain an adequate number of specimens. For this reason the data based on the records made at the time of transplantation will be shown in Table VIII. On account of the fact that the records were taken on the examination of the seedlings, which extended to only two or three leaves, I failed to note the heart leaves as distinguished from the normals. So the normal class may contain heart leaves in the families segregated for their factor. The F_2 results prove the occurrence of segregation of two recessive factors, the "Rangiku" and the willow, as may be seen in the F_2 data. The genetic composition of the parental willow and "Rangiku" plants are considered to be $w_w w_w hhII$ and $W_w W_w HHii$ respectively, where w_w denotes the willow, h the heart and i the "Rangiku." On the triple combination of such factors the F_2 generation may be expected to be composed of:

Genotype	Its ratio	Phenotype	Its ratio
$W_w W_w HHII$	1	Normal leaf	9
$W_w W_w HHii$	2		
$W_w W_w hhII$	2		
$W_w W_w hhii$	4		
$W_w W_w HhII$	2	Roundish normal leaf	18
$W_w W_w Hhii$	4		
$W_w W_w hhII$	4		
$W_w W_w hhii$	8		
$W_w W_w hhII$	1	Heart leaf	9
$W_w W_w hhii$	2		
$W_w W_w hhII$	2		
$W_w W_w hhii$	4		
$W_w W_w HHii$	1	"Rangiku" leaf	12
$W_w W_w Hhii$	2		
$W_w W_w hhii$	2		
$W_w W_w hhii$	4		
$W_w W_w hhii$	1		
$W_w W_w hhii$	2		

Genotype	Its ratio	Phenotype	Its ratio
$w_w w_w H H I I$	1	Willow leaf	9
$w_w w_w H h I I$	2		
$w_w w_w H H i i$	2		
$w_w w_w H h i i$	4		
$w_w w_w h h I I$	1	Roundish willow leaf	3
$w_w w_w h h i i$	2		
$w_w w_w H H i i$	1	Split willow leaf	4
$w_w w_w H h i i$	2		
$w_w w_w h h i i$	1		

The hypothesis offered above is made on the basis of the results, so no further discussion on the subject is necessary.

TRIPLE SYSTEM OF ALLELOMORPHS.

As was fully stated in the foregoing pages the willow leaf behaves as a recessive to the normal, three-lobed leaf. But curiously enough, it gives almost habitually, though rarely in frequency, the maple bud-sports (Pl. X, figs. 10 and 13). Why has the willow leaf a tendency to change towards the maple? Is the willow leaf allelomorphic to the maple? Also, what relation is there among the normal, the maple and the willow leaves? Both leaf forms, maple¹ and willow, act as recessive characters to the normal. If we assume tentatively two recessive factors denoted by *a* and *b* respectively, the genetic composition of the normal leaf should be **AABB**, while both maple and willow leaves, each of which constitutes a monohybrid in hybridization with the normal, may have **aaBB** and **AAbb** compositions respectively. If this were the case, what sort of transformation of the willow leaf factor might occur in the continuously sporting phenomena? It is out of the question to think that **AAbb** might have changed at one step into **aaBB** or **aaBb**. So there is no other conclusion but that the genetic composition of the maple sport on the willow strain is **aabb**. Such a constitution may be considered as the result of transformation of the two **A** factors to the **aa**-condition. The sport maple branches and individuals, however, produce no seeds, so we could not examine the genotype by their descendants. When the willow leaf, however, is crossed with the maple the F_1 should revert to the normal as the result of recovery of both dominant factors, **A** from the willow parent and **B** from the maple partner. Such F_1 may produce offspring composed of 9 normals (**1AABB** + **2AaBB** + **2AABb** + **4AaBb**): 3 willows (**1Aabb** + **2Aabb**): 4 maples (**1aaBB** + **2aaBb** + **1aabb**) in every 16 individuals. To see whether this is the case or

¹ Toyama, K., "On one or two Mendelian characters," *Journal of the Japanese Breeders' Association*, vol. 1, No. 1, 1916, in Japanese. Imai, Y., "Genetic studies in Morning Glories. II," *Botanical Magazine, Tôkyô*, vol. xxxiv, 1920, in Japanese.

not, I attempted to fertilise the common maple flowers by the pollen of the willow flower, and five individuals were raised from the seeds thus crossed. The F_1 plants, contrary to expectation, all bore maple leaves and bloomed with five-split flowers. Among them, three plants were allowed to grow for the purpose of obtaining seeds, but they proved to be sterile. The leaf form of the F_1 plants was maple, but the figure was somewhat more slender than the common maple. The narrow lobed maple was also a characteristic of the maple sport on the willow specimens. From these facts we may conclude that the normal, maple and willow leaves make up a triple system of allelomorphs. The order of dominancy is normal \rightarrow maple \rightarrow willow, and the last has a tendency to mutate into the middle form. Let us now attempt to represent the factors for these three leaves in one series of allelomorphs, namely, **M**, **m** and **m'** respectively. The occurrence of the maple sport on the willow strain should be, then, due to the result of the factor mutation from the willow, **m'm'**, to the heterozygous maple, **mm'**, and the slender figure of the sporting maple leaves may be understood to be the result of the recessive factor. The phenomena of multiple allelomorphs are not rare in the Japanese Morning Glory. I have detected five cases of a triple system of allelomorphs in this plant, viz.:

1. Normal, maple and willow leaves.
2. Normal, cup flower with crapy leaf and cup flower with normal leaf¹.
3. Fully coloured, "Shedding Tears" and "Circular Cloud" (colour patterns of corolla).
4. Normal, common contracta and contracta with "platicodon" flower.
5. Black, brown and tan seeds (?).

MUTABILITY OF THE WILLOW FACTOR TO THE MAPLE.

In the various crosses of the willow leaf as was shown in the previous tables we had almost invariably a few maples (Pl. X, figs. 9, 11 and 12) among the expected segregates, besides the rare occurrences of the maple bud-variation in the willow plants. So the spontaneous occurrence of the maple leaf may be considered a common phenomenon in the segregating families of the willow leaf. In Table X (p. 95) the available

¹ Imai, Y., "Genetic studies in Morning Glories. XI. On the variegated and the heart leaf linkage groups in *Pharbitis Nil*," *Botanical Magazine*, Tôkyô, vol. xxxviii, 1924, in Japanese.

data, which I now have on hand, will be found collected and summarised. Among them, the F_3 data of the cross, 65×505 , were obtained from two sources, the one from Table IV, and the other from Table IX which consists of the records made at the seedling stage of the other families besides those given in the former table.

Thus the numerical proportion of the willow and non-willow leaves is nearly 1 : 3, but it contains about 0.4 per cent. of the maple individuals and approximately 0.12 per cent. of the maple bud-sports among the willows. The fact that the spontaneous appearance of the maple rogues and bud-sports is limited to the willow crosses tells us of an intimate relationship between the maple sports and the willow leaf. On a consideration of the spontaneous appearance of the maple, the occurrence of the bud-sports seems to give us a clue to the solution of the problem. The sport branch bears the maple leaves and blooms into five broad petals like that known as the "maple flowering" (Pl. X, fig. 10), while the willow parts of the same individual remain quite normal for the willow leaf and the five narrow lobed petals. Such bud-sports were detected on three plants among 1796 willows. The total comprises all the available specimens except that a part of the F_3 data given in Table IX is omitted on account of the inadequate conditions obtaining for our purpose, in that the record was taken from a bed of seedlings not fully grown, though even with such a handicap I observed one bud-sporting specimen. As was shown in the previous section the maple leaf acts as the second member of the triple allelomorphs of the willow series, so that its genetic difference to the willow must be in one factor. So the bud-sport may be considered to be the result of vegetative mutation of m' to m . Actually the willow leaf carries the $m'm'$ -composition and as the result of its transformation to the mm' -composition the maple branch may be produced. Such sporting individuals being in the proportion of 0.17 per cent. among the total willows, the frequency of the vegetative transformations of the willow to the maple must be rare, though the phenomenon is habitual. As to the individual sports 61 maple mutants are formed among 15,200 observed individuals, the frequency of the occurrence being 0.40 per cent. The ever-sporting nature of the willow factor to the maple in the vegetative tissues may indicate the possibility that these mutants are at least partly due to the result of the vegetative sport occurring on the mother plant. If this were the case, the seeds collected from the non-willow plant which looks quite normal, but contains mutated tissue of the Mm -composition, should give some maples among the expected segregatives. The proportion of

TABLE IX.

*The F₃ data of the cross 65 × 505.
 Offspring of the non-willow leafed F₂.*

Pedigree No.	Non-willow leaf	Willow leaf	Maple leaf	Total
Total of 35 pedigrees	3461	—	—	3461
59	43	19	2	64
60	5	4	—	9
61	30	9	—	39
62	84	24	1	109
63	27	8 ⁽¹⁾	2	37
64	45	21	—	66
65	100	27	—	127
66	59	19	1	79
67	13	6	—	19
68	201	55	—	256
71	73	31	—	104
72	21	4	—	25
73	32	6	—	38
74	124	44	1	169
75	48	23	—	71
76	66	22	—	88
77	98	42	—	140
78	128	39	—	167
79	27	5	—	32
80	62	13	2	77
82	51	18	—	69
83	75	28	1	104
84	34	7	—	41
85	27	13	—	40
86	144	31	—	175
94	25	6	1	32
95	137	38	—	175
97	60	17	—	77
100	71	24	—	95
103	101	45	—	146
106	16	9	2	27
107	165	44	2	211
109	284	91	3	378
110	43	11	—	54
111	3	2	1	6
117	194	50	1	245
119	89	22	2	113
120	54	25	1	80
121	59	14	—	73
122	44	8	—	52
124	4	1	—	5
125	80	29	1	110
126	131	39	—	170
127	17	7	—	24
128	25	11	—	36
130	29	16	—	45
131	73	29	1	103
133	84	29	—	113
136	17	5	—	22
138	160	43	—	203
142	297	75	3	375
144	73	20	—	93
145	104	19	1	124

TABLE IX (continued).

Pedigree No.	Non-willow leaf	Willow leaf	Maple leaf	Total
146	55	21	—	76
147	37	12	—	49
148	30	8	—	38
149	68	24	—	92
150	67	18	—	85
151	240	64	—	304
152	97	20	—	117
153	33	9	—	42
154	140	37	—	177
155	49	13	—	62
156	112	29	1	142
157	17	4	—	21
158	75	26	—	101
159	175	41	4	220
160	106	37	3	146
162	46	11	—	57
Total	5403	1621 ⁽¹⁾	37	7061
Expected	5295.75	1765.25	—	7061

The numeral in brackets denotes the number of the plants which made bud-variation.

TABLE X.

Cross	Non-willow	Willow	Bud-Sporting willow	Maple	Total
Y 120	93	34	—	2	129
Y 122	38	9	—	—	47
Y 123	210	70	1	—	281
Y 120—(1-4)	45	13	—	—	58
65 × 505— F_2	309	73	—	4	386
— F_3 (from Table IV)	2481	725	2	12	3220
— F_3 (from Table IX)	5403	1620	1	37	7061
326 × 505— F_2	204	56	—	—	260
— F_3	2269	615	—	1	2885
M 3 × 505— F_2	62	23	—	1	86
— F_3	608	175	—	4	787
Total	11722	3413	4	61	15200
		3417			
		3478			
Expected	11400	3800			15200

the appearance of the maple mutants may vary according to the degree of the extent of the area of the mutated tissue. Generally speaking, the result of such a vegetative mutation is to cause the mutants to occur more or less in a mass. - Actually we observed no adequate positive evidence in the segregating families of the divergent crosses. Examining carefully the actual numbers of the tables we see that the mutants generally occur one by one in each family. For this reason the possibility

that the mutants are due to the result of somatic variation in the tissue of the parental plant seems to be small. So we may recognise that the origin of maple mutants, at least in the majority of cases, lies in the gametogenesis¹ of the willow plants. But even if we can hardly recognise the possibility of the somatic occurrence of the mutation as a cause of the appearance of the maple mutants, this does not mean that we altogether abandon the expectation of such an actual occurrence. As was stated before, we had 0.17 per cent. occurrence of bud-sports among the willow leaves. So it is not impossible to expect such a somatic variation also on the **Mm'**-individuals. Practically, however, even if this were so, the frequency of such an occurrence would be low, only about one case in every six hundred trials, so we cannot be far wrong in taking for granted that the consideration of such occurrence may be placed outside the problem.

Let us now attempt to make a quantitative estimate of the frequency of the occurrence of the mutation, **m'** → **m**. As was stated, we can recognise that the maple mutants are possibly due to the result of gametic mutation. If we regard all the maple mutants to be originated by factorial change in gametogenesis the following calculation may be made: In the normal leaves which are heterozygous for the willow factor two sorts of gamete, **M** and **m'**, would be expected in an equal ratio. Actually, however, the contents of gametes are complicated by the ever-sporting occurrence of the willow factor in the maples. Let us represent the frequency value of the mutation, **m'** → **m**, by x , and denote the total of the gametes as 2, then, the contents of the gametes actually produced must be supposed to be $1\mathbf{M} + (1 - x)\mathbf{m}' + x\mathbf{m}$, namely in the ratio of 1 normal gamete : $1 - x$ willow gamete : x maple gamete. On sexual combination of these three sorts of gametes we should expect to obtain the following zygotes:

$$\{1\mathbf{M} + (1 - x)\mathbf{m}' + x\mathbf{m}\}^2 = 1\mathbf{MM} + 2(1 - x)\mathbf{Mm}' + 2x\mathbf{Mm} \\ + (1 - x)^2\mathbf{m'm}' + 2(1 - x)x\mathbf{m'm} + x^2\mathbf{mm}.$$

The frequency value of the occurrence of the maple mutation, then, may be calculated on the basis of the above formula. By the formula we understand that the respective frequency of the homozygous normal, the heterozygous normal for the willow, and the heterozygous normal for the maple is in the proportion of $1 : 2(1 - x) : 2x$. If the examined total numbers, the sum of the data of Tables IV, VI, VIII and IX, are applied to the above ratios the following formulae may be deduced:

¹ My opinion on this view will be stated in a future paper.

$$\frac{MM}{Mm'} = \frac{1}{2(1-x)} = \frac{82}{179} \dots\dots\dots \text{Formula 1,}$$

$$\frac{MM}{Mm} = \frac{1}{2x} = \frac{82}{1} \dots\dots\dots \text{Formula 2,}$$

$$\frac{Mm'}{Mm} = \frac{2(1-x)}{2x} = \frac{179}{1} \dots\dots\dots \text{Formula 3.}$$

As Formula 1 gives a minus answer, this case may be placed outside this calculation. From Formula 2 we get the result of 0.0061, and from Formula 3 that of 0.0056, a very similar value. On the basis of these values a frequency of mutation of the $m' \rightarrow m$ of about one in every one hundred and seventy gametes may be expected. Another calculation may be made on the basis of the numbers of the maple and willow leaves. The formula is:

$$\frac{mm + mm'}{m'm'} = \frac{x^2 + 2(1-x)x}{(1-x)^2} = \frac{61}{3417} \dots\dots\dots \text{Formula 4.}$$

On account of the fact that we cannot distinguish¹ the mm -carrying and the mm' -carrying maples, because they produce no seeds, two genotypes are considered together in the calculation of this formula. The answer obtained from this formula is 0.0091, showing a value approximating those calculated from Formulas 2 and 3. Critically speaking, the former results are more reliable than the latter; for since the latter were calculated on the basis of a single plant of the Mm -class they have only a rough value. For this reason the frequency of the mutation of the $m' \rightarrow m$ may be considered to be 0.0091, or 0.91 per cent. When we consider, however, the respective percentage of the mutability in the different crosses it varies to a considerable extent as will be seen from the subsequent table:

TABLE XI.

Cross	Willow leaf	Maple leaf	Total	Mutability
Y 3 pedigrees	127	2	129	0.78 %
65 × 505	2421	53	2474	1.08 %
326 × 505	671	1	672	0.07 %
M 3 × 505	198	5	203	1.24 %
Total	3417	61	3478	0.91 %

In short, the frequency varies in extent from 0.07 per cent. to 1.24 per cent. Such a considerable variation of the values in the different

¹ Practically, only one case in every one hundred and eleven maple mutants may be expected in the homozygotic condition, if we calculate the proportion on the basis of 0.9 per cent. as the frequency for the maple mutation.

crosses, it would seem could hardly be attributed to the chances of sampling. I am inclined to think it is the result of the modifier or modifiers which influence the frequency of the mutability. This hypothesis, however, must be tested by future study.

I wish to take this opportunity to express my hearty thanks to Prof. K. Miyake, under whose direction the experiments were conducted, and to Mr K. Hashimoto for the substantial encouragement he so kindly gave. Last but not least I would also express my cordial thanks to Messrs B. Kanna and K. Tabuchi for their friendly help in my investigation. Without these gentlemen the completion of the present study might have been doubtful.

SUMMARY.

1. The willow leaf acts as a recessive to the normal.
2. The willow factor (m') represents the narrow-lobed cotyledon and the willow leaf as well as the narrow split corolla.
3. The factor for the normal, maple and willow leaves may constitute a triple system of allelomorphs.
4. The order of dominancy of these factors is the normal (M) \rightarrow the maple (m) \rightarrow the willow (m').
5. The willow factor produces the roundish willow leaf in combination with the factor for the heart leaf.
6. The filamentous willow leaf is produced as a combined result of the factors for the willow and the "Sasa" leaves. The flower of this strain blooms into a five-lobed filamentous corolla.
7. On combining the factors for the willow and the "Rangiku" leaves we get the split willow leaf, which blooms into a flower with characteristics of both factors; namely the flower is creased and split.
8. Maple bud-sports were rarely, but habitually, observed on the willow plants.
9. The frequency of the occurrence of such sporting plants among the willows is estimated to be 0.17 per cent.
10. More frequently maple rogues appeared in the segregating families for the willow leaf.
11. The origin of these variations, both bud-sport and individual rogue, lies in the result of mutation of the $m' \rightarrow m$.
12. The maple rogues seem to originate, at least in the majority of cases, through the occurrence of mutation in gametogenesis.
13. The frequency of the gametic mutation of the $m' \rightarrow m$ is 0.91

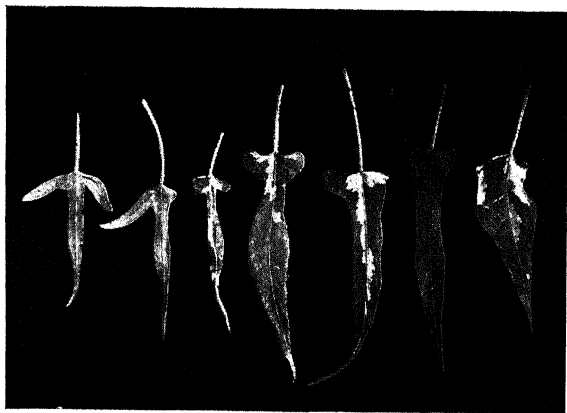


Fig. 1.



Fig. 2.

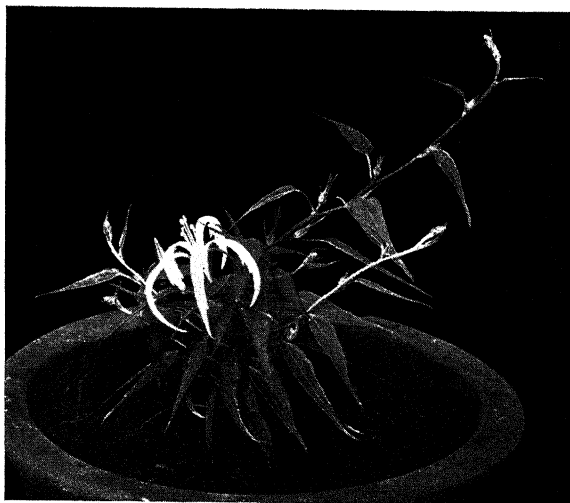
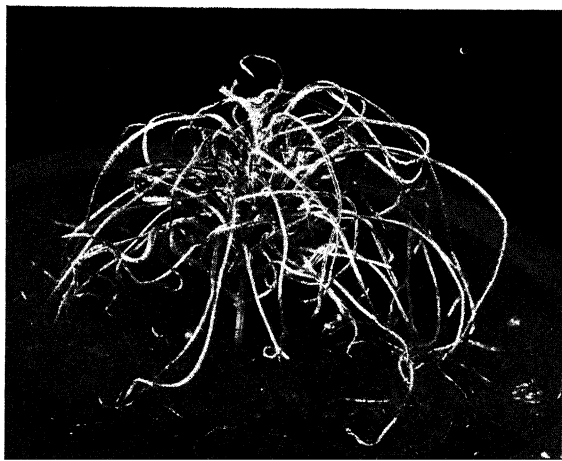


Fig. 3.



Fig. 4.



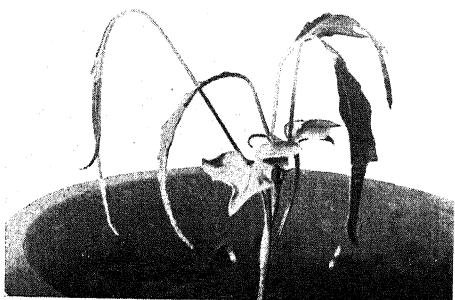


Fig. 7.

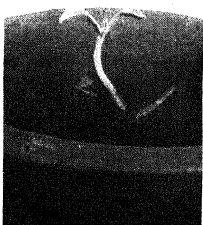


Fig. 8.

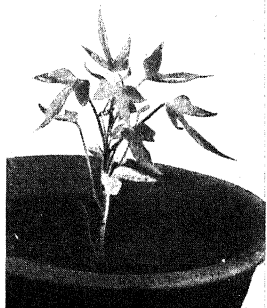


Fig. 9.



Fig. 10.



Fig. 12.



per cent., showing that there is about one chance of such an occurrence among one hundred and ten willow gametes.

14. The frequency, however, varies considerably in the different crosses.

15. The willow leaf invariably produces no seeds. This is also true in the maple mutants, which are considered to be heterozygotic for the willow factor.

EXPLANATION OF PLATES IX, X.

- Fig. 1. Variation of the common willow leaf.
- Fig. 2. Seedling of the roundish willow bearing punched leaves.
- Fig. 3. Blooming roundish willow plant.
- Fig. 4. Slender three-lobed willow specimen, an extreme form of the normal willow.
- Fig. 5. Blooming roundish willow cultivated in the field.
- Fig. 6. Filamentous willow specimen.
- Fig. 7. Seedling of the split willow specimen with irregular-shaped cotyledons.
- Fig. 8. Seedling of the split willow with cotyledons in the shape of tiny spatula.
- Fig. 9. Seedling of a maple mutant with slender three-lobed leaves.
- Fig. 10. A willow bearing a maple bud-sport. Note the marked contrast in foliage and flower.
- Fig. 11. A portion of the stem of a maple mutant with five-split flower.
- Fig. 12. Heart-shaped maple mutant with the "maple blooming" flowers.
- Fig. 13. A roundish willow leafed seedling making a vegetative variation toward the heart-shaped maple. Note the two largest leaves which are a mosaic of half willow and half maple.

ON CERTAIN ABERRATIONS OF THE RED-LEGGED PARTRIDGES *ALECTORIS RUFa* AND *SAXATILIS*.

By W. BATESON, M.A., F.R.S., AND
GREGORY BATESON (*Scholar of St John's College, Cambridge*).

(With Four Coloured Plates.)

INTRODUCTION.

IN 1923 Mr P. R. Lowe, Assistant Keeper in the British Museum Bird Room, brought to my notice two very striking and quite distinct aberrations of the Red-legged Partridge (*Alectoris rufa rufa*) which the Museum possessed. The first, which we call the "bright" variety, was represented by three specimens which all came from localities not very far removed from each other in the west of England; and of the second, which we call the "dull" variety, there were also in the collection three specimens, two from Kent, having been shot in the same field in successive years, and a third from Essex. Subsequently we came to know of another specimen sent by Lord Deerhurst to Rowland Ward's for mounting, which though presenting certain differences agreed in many respects with the "bright" birds. On hearing of the interest attaching to it Lord Deerhurst very kindly presented the bird to the Museum. We learnt also from him that the bird was one of several similar birds which had been observed in his neighbourhood during a period of years. This locality (Pirton in Worcestershire) is moreover not far from those of the other "bright" birds.

Exact details respecting the origin of the several specimens of both varieties are given below.

Briefly the distinguishing features of the "bright" variety as represented by the first three specimens (Lord Deerhurst's bird being for the present omitted from consideration) are as follows:

1. Head *black*, not reddish brown.
2. Feathers of mantle, scapulars and wing coverts in various degrees *barred*, in such a way that many of them repeat the chevron marks so conspicuous in the flank feathers of the normal.
3. Tail coverts *bright chestnut brown* like the normal rectrices.
4. Whereas the normal has the throat a clear white, limited posteriorly by a solid black band or "gorget," in the bright variety this sharp

differentiation is diminished, and the throat is a dingy grey, with a thin band of broken colour in the place of the gorget.

Lord Deerhurst's bird differs from the three bright birds in being much paler in colour, and in the fact that the head is *not black*, but as regards the features numbered 2, 3 and 4 it agrees with them fairly well. This bird is evidently immature, and with some probability its special peculiarities may be ascribed to juvenility.

The characteristics of the "dull" variety are:

1. The *throat* is *black*.
2. The *chevron marks* of the normal flank feathers *are altogether absent*.
3. The *rectrices* are not chestnut brown as in the normal but are of the same *dull colour* as the tail coverts.
4. The dorsal plumage is much as in the normal, except that the feathers of the neck are little differentiated; but the ventral surface is altogether peculiar, being, except for the black throat, of a more or less unicolourous brown. This brown is reddish anteriorly on the part of the breast which in the normal is ashy blue. The flanks and belly are a dull brown, broken by a central patch of irregularly placed white feathers just posterior to the breast.

Minor distinctions will be specified in the detailed descriptions.

So far as we are aware the only published reference¹ to either of these varieties of *A. rufa* is a brief account given of the "bright" variety by Mr Lowe in the *Field*, 15 March, 1923, illustrated with a black and white sketch by Mr Frohawk.

From a mention by Ogilvie-Grant² we learned however that Fatio had recorded a variety of another species, *A. saxatilis*, as characterised by a *black head*, which is so conspicuous a feature of our bright variety. A description of two specimens showing this feature was published by Fatio³ with a black and white plate showing the general appearance of the variety and a plate in colour giving representations of a number of feathers. Obviously the variety *melanocephala* strongly resembles our bright birds. As these birds had been presented to the museums of Sion and Bex my son went to Switzerland to examine them in detail, which by the courtesy of the curators he was permitted to do. He was also successful in finding in the Geneva Museum another specimen of *A. saxatilis* which though approximately normal had a few of the

¹ For earlier references see note at end of the paper.

² F. M. Ogilvie-Grant, *Handbook to the Game-Birds*, 1895, I, p. 91. Allen's Naturalists' Library. Ed. R. Bowdler Sharpe.

³ V. Fatio, *Perdix saxatilis* var. *melanocephala*: curieux déplacements de Couleurs. *Mem. Soc. Zool. France*, 1894, VII, p. 393.

abnormal barred feathers in the scapular region. He has prepared the following account of the whole series, including our three bright birds, Lord Deerhurst's bird, the three dull birds—all *Alectoris rufa rufa*; the two bright birds at Sion and Bex respectively, the peculiar specimen at Geneva—all *Alectoris saxatilis*; together with observations made on a considerable number of birds of both species which may be regarded as normal.

If scientific names are required for the designation of these aberrations we should follow Fatio in referring to the bright variety in both species as var. *melanocephala*, and to the dull birds we may give the name var. *obliterata*. W. B.

DESCRIPTION OF MATERIAL.

LIST OF SPECIMENS.

Alectoris rufa rufa var. *obliterata*.

(Fig. A, Plates XI and XII.)

This form, which is referred to as the "dull" variety, is represented by three specimens, all of which are in the British Museum. Their data are:

- (1) ♂, from Spaynes Hall, Braintree, Essex; shot 20 Oct. 1908.
Presented by A. W. Ruggles Brise, Esq. Plates XI and XII, A.
Reg. No. 1908. 10. 22. 1.
- (2) ♂, from Higham, near Gravesend, Kent; shot 1 Sept. 1914.
Presented by Dr Hammond Smith.
Reg. No. 1915. 1. 15. 1.
- (3) ♂, from Mockbeggar, Rochester; shot 1 Sept. 1915.
Presented by Herbert Cobb, Esq.
Reg. No. 1915. 10. 5. 1.

The label states that (3) was shot in the same field as (2). This is as a matter of fact in accord with the names of the locality, which may well be synonymous.

As far as we know, no other specimens of the var. *obliterata* have been seen.

Alectoris rufa rufa var. *melanocephala*.

This form is generally referred to in the paper as the "bright" variety. There are four specimens in the possession of the British Museum. Their data are:

Red-legged Partridges

- (1) ♂, from Overbury, Worcestershire (near Bredon Hill); shot 9 Oct. 1903.

Presented by Wynyard Warner, Esq.

Reg. No. 1904 . 10 . 2 . 1. Figured by Lowe, *Field*.

- (2) Old ♂, from Norton Hall, Gloucestershire (near Gloucester); shot 26 Oct. 1922. Plates XI and XII c.

Presented by Major Robert Bruce.

Reg. No. 1923 . 1 . 29 . 1 (genitalia preserved).

- (3) From Kinver, near Stourbridge, Worcestershire; without spurs; ? ♀. 1923. [No date.]

Presented by A. H. Guest, Esq.

Reg. No. 1923 . 3 . 11 . 1.

- (4) Juvenile ? ♀, from Hermitage Farm, near Pirton Court, Worcester.

Shot 21 Sept. 1923 and presented by Viscount Deerhurst.

This bird differs from the others in numerous points and therefore has been described separately.

Besides these four specimens which are preserved in the British Museum, Lord Deerhurst records that in 1919 a covey of partridges near Pirton Court contained three birds probably all of this type. One of these was shot to pieces but the other two escaped and were never seen again. In 1922 he himself shot a brightly coloured partridge in the same part of the same field as the specimen which he shot a year later and gave to the Museum (No. 4 in the list). Thus in all there are three specimens of the dull birds and four of the bright birds. The three dull birds are all *males* and all from the east of England, the four bright birds are *males* with the exception of Nos. 3 and 4, which are doubtfully females; all four are from the west of England, where four other similar birds have been seen.

This distribution of the two varieties is remarkable, and since red-legged partridges are so frequently shot, there is no doubt that the two varieties are almost confined to the respective areas as we know them.

Alectoris graeca saxatilis.

No specimens comparable with the dull birds are known. There are three specimens which are comparable with our bright variety of *A. rufa*. These are:

Alectoris graeca saxatilis var. *melanocephala* Fatio.

- (1) Juvenile, 17 November, 1878, shot close to Sion in the Canton de Valais by Alphonse Bonvin, who told Fatio that it was the only

abnormal bird in a covey of eight, five of which were shot. This specimen is now in the Musée de l'École Scientifique at Bex in Valais.

(2) ♀ adult, 11 December, 1879, shot in the Val d'Herens, near Sion, by A. Bonvin. This bird is in the Musée Cantonal at Sion.

(3) A bird in Geneva University Museum which differs from the normal notably in possessing *a few barred dorsal feathers*. It has the following data: No. $\frac{763}{44}$, ♀ Orsières, Valais, November 1912, presented by M. Ghidini.

This bird is separately described.

Thus all three of these "bright" birds were taken in the same canton of Switzerland within a radius of thirty miles. (1) and (2) are substantially alike, but (3) approximates to the normal except in the fact that it has some barred feathers on the back.

DESCRIPTION OF THE FORMS.

Alectoris rufa. "Dull" variety or var. *obliterata*.

Dorsal and ventral views of this form are shown in Plates XI and XII A; separate feathers in the bottom row of figures in Plate XIII. The three specimens are all very much alike, and differ strikingly from the normal birds in the loss of all those contrasts of colour which make up the decorated appearance. This is most noticeable in the flank feathers, which, instead of being brightly coloured and ornamental as in the normal (Plate XIII 1, B), are grey at their bases with brownish grey tips (Plate XIII 1, A). Correspondingly the rest of the body is dull and uniformly coloured. The feathers of the breast which are ashy in the normal are tipped with brown, matching the dorsal and flank feathers. The abdominal feathers are similar and only very slightly paler than those of the back.

The tail feathers instead of being chestnut are a dull greyish brown with spots of darker pigment, but otherwise match the tail coverts. This last point is especially worth notice since in the bright variety the coverts and rectrices match, but there the uniformity is brought about by a change in the colour of the coverts, not of the rectrices as in the dull birds.

The primary flight feathers are almost normal, possessing still some traces of the pale streak in the normal feathers near the tip. But this streak is much reduced on the more internal primaries and is completely absent from the secondaries.

The only contrasts of colour are provided by the throat and by the irregular white patches (present on all three birds) on the lower breast.

The throat is black and these black feathers are followed by patterned feathers comparable with those on the lower neck of a normal bird but much less conspicuous. The pattern consists of black marks upon a dull brown ground, not on the white and grey ground of the normal. The similarity of the patterns on the neck feathers of the type and the variety, and the fact that in each case these patterned feathers are preceded by a black area, make it appear as if the factors which are responsible for the black area and for the patterned feathers had changed their sphere of influence, producing similar colours more anteriorly on the neck, obscuring the white throat and leaving their old areas to the mixed grey and chestnut pigments which make up the dull brown of the remainder of the bird. On the dorsal side of the neck there are some patterned feathers, which feathers bear the same patterns and occupy approximately the same area as on the normal, but again the pale background has been replaced by dull brown.

Before leaving these birds, for the sake of completeness I should add that specimen No. 1 has a single scapular feather with a black fringe at its tip, and that the same bird has a single abdominal feather much paler than the others—dirty yellow with black markings on it which form an imperfect V of which the point is directed outwards.

Specimens Nos. 2 and 3 have each of them one or two irregular feathers. On one of these birds (Mockbeggar) there is a feather with a brown mottled centre among the feathers of the white patch. Another breast feather of the same bird has an irregular black mark on each side.

Alectoris rufa. "Bright" variety (Plates XI and XII c).

Of the four specimens of this type three are nearly alike; the fourth is very much younger than the others and will be described separately, since some of its juvenile characteristics have a bearing upon the variation of *Alectoris saxatilis*.

The three similar birds have the following characteristics:

The dorsal side of the head (*i.e.* the cap), which in normal birds is greyish brown with very small patches of black hairlike feathers round the base of the bill, is uniformly black. The concealed bases of the feathers are pale grey.

The details of distribution of black pigment in the cap of normal and abnormal birds are perhaps worth noticing:

In the normal there are little hairlike black patches laterally at the base of the bill and smaller patches dorsally and ventrally at its insertion.

(These patches are also present in *A. saxatilis*, in which species they are more clearly developed than in *A. rufa*.) They are followed posteriorly by a few feathers with black bases and grey tips; *i.e.* there is an antero-posterior series of feathers on which, as we proceed backwards, the black is seen to be more and more closely confined to the base of the feather, until after a few feathers it is present only as a trace at the base of the feather and finally disappears. This arrangement of pigments is very typical of the colours of feathers, and in this paper we shall speak of it as the *basipetal disappearance* of colour. Another similar case will be described in the black collar band of normal *A. saxatilis*.

In the abnormal birds the arrangement of black pigment is very different. The base of the bill is, as in the normal, surrounded by patches of short black hairlike feathers, but these are succeeded posteriorly by feathers which are greyish white at the *base* and black at the *tip*. Behind these the area of the black increases and soon covers the whole feather, until it is displaced by grey which starts at the tip and spreads downwards, covering a larger and larger area as we proceed down the median dorsal line of the neck. In this region the series is complicated by some patterning of which there is no need to give details beyond stating that this patterned region corresponds to a similar larger region on the neck of the normal, where the patterns are substantially the same but more conspicuous on account of the pale grey-and-white ground colour. In the Overbury bird, however, the laced feathers of the gorget are present also dorsally, forming a central band on the back of the neck.

This description of the head holds good for the Norton Hall bright *A. rufa* and for the adult *A. saxatilis*, but in the case of the juvenile *A. saxatilis* and of the Kinver and Overbury birds there are many feathers on the top of the head which are black with a greyish chestnut fringe. This fringe shows little serial change in breadth, and so must be regarded as some form of barring. (Cf. p. 113.)

Turning now to the ventral side, in the normal bird the throat is white, behind it is a heavy black band (gorget) which grades off into patterned grey, black and white feathers. In the bright variety the throat is not white but a dirty pale grey, the heavy black band is absent and the area of the patterned feathers much reduced. This condition resembles closely that of young normal *A. rufa*, in which the black band does not appear until after the patterned feathers are formed (cf. description of the juvenile bright bird in which absence of black on the head is ascribed to its immaturity).

Though dorsally there was no change in the patterns of the neck

feathers, they being apparently simply streaked with a black longitudinal line on each side as in the normal, ventrally there is a considerable change, which is shown in Plate XIII, figs. 8 b and c. In the normal the black is segmented by transverse invasions of greyish white; in fact the feather may be regarded as transversely barred. Proceeding posteriorly in the series the proximal segments of black fade away while fresh segments are separated off from the large black spots at the tip. In the bright variety there is no trace of this segmentation. The feathers have pale yellow centres and black margins. Feathers of this type are also found in the *nuchal* region of the Overbury bird.

The remainder of the ventral side of the bright variety is normal. The flank feathers which are by accident partially hidden in Plate XII c are normal¹, as also are the grey feathers of the breast and the yellow abdominal feathers.

The feathers of the back and wings remain to be described. These are most conveniently considered as in three separate systems:

(a) Median dorsal group, extending from the insertion of the neck back to the rectrices. In this group the term "mantle" is used for those feathers which are inserted on the back between the two scapular pteryloses.

(b) Scapular feathers.

(c) Wing coverts and remiges.

(a) *Median dorsal group of feathers.*

Feathers of the mantle are shown in Plate XIII, fig. 4 c and in Plate XIV, fig. 3. The more lateral feathers of the group are strongly curved and the barring of the normal flank feathers is very closely reproduced, but the pattern has become asymmetrical, giving, instead of a transverse bar, a chevron mark which is best developed on the outer side of the feather. Among these feathers are a few on which the bar is apparently doubled—Plate XIV, fig. 12. In these a second black bar has appeared proximal to the first and separated from it by the pale bar which is present on normal flank feathers and on the neighbouring mantle feathers. The pattern so arrived at is comparable with that of the normal flank feathers of *A. saxatilis*, on the flanks of which the proximal black bar is always well developed (Plate XIV, fig. 10). Occasionally, too, this bar is faintly developed on the extreme anterior flank feathers of normal *A. rufa* (see Plate XIV, figs. 13 and 14),

¹ The differences in the flank feathers shown in Pl. III, figs. 1 b and c, and Pl. IV, figs. 5 and 6, are probably due to the feathers having been taken from slightly different parts of the pterylosis.

so that it is by no means surprising that this bar shows itself in the mantle feathers of the variety.

The more central feathers of the mantle are symmetrical (Plate XIII, fig. 4). They are pale feathers with a broad dark V on the fan; outside the V is a paler fringe. Behind the mantle on the back the dark mark is broader and blacker (Pl. XIII, fig. 5 c) and is on some feathers partially split into two transverse bars by formation of an island of chestnut. But the division never proceeds very far in this region of *A. rufa*.

Following the median dorsal series backwards, apart from the tendency to divide, the bar does not change its position on the feather. It becomes paler on the posterior part of the back and finally fades away in place—it does not disappear basipetally like the black of the head feathers. (See Plate XIII, figs. 4, 5 and 6 c.)

The black still persists as a faint mark near the tip of the rump feathers (so faint that it does not show in Plate XIII c, fig. 6). But one of these feathers on the Norton Hall bird has a distinct elliptical black line, the long diameter of the ellipse coinciding with the rachis. The centre of this ellipse is filled with chestnut. This feather is quite isolated and no doubt indicates a stage in the development of the broad black bar and its partial splitting.

The tail coverts are almost self-coloured with a darker chestnut tip on a pale chestnut ground. These shade off gradually in colour to the dark chestnut of the rectrices.

In the normal bird, too, the rectrices are chestnut, in marked discontinuity with the grey of the rump feathers and tail coverts. Apparently, then, the normal self-coloured bird has been subjected to some factor which left the rectrices unaffected. When this factor was removed (in the bright variety) the feathers of the rump were left in continuous series with the rectrices, no longer contrasting with them. On this hypothesis the grey rectrices of the dull bird would be due to an increase of the area subjected to the dulling factor¹. This and other similar suggestions will be discussed more fully at the end of the paper.

(b) *Scapular feathers.*

The anterior scapular feathers resemble those of the mantle in reproducing the patterns of the flank feathers of the normal bird, but among them I have not seen any on which the proximal bar has reappeared giving a false appearance of doubling. Among the posterior

¹ Obviously this representation might be inverted, the dull var. being regarded as due to loss, and the bright var. to addition. Between these two hypotheses we cannot decide. Whichever be adopted, the normal is the middle term.

scapulars there is another and quite distinct type of doubling (Plate XIV, fig. 15) not seen on normal flank feathers. In this process the proximal bar has split, or been invaded by the chestnut of the fringe, so that the two resulting bars are separated, not by a pale bar, but by chestnut. Beyond the distal bar is a white fringe. In Fig. 15 a still further splitting is indicated.

The homologies of the bars may be tentatively summed up in tabular form:

<i>rufa</i> flank	<i>saxatilis</i> flank	<i>rufa</i> var. C mantle	<i>rufa</i> C posterior scaps. (Inner web)	<i>saxatilis</i> posterior scaps. (Inner web)
Grey base	Grey base	Pale grey	Yellow	Grey
Chestnut	Grey	Pale yellow	Chestnut-yellow	Chestnut-yellow
Grey	Grey	Grey	Grey	Grey
<i>Absent</i>	Black	Black	<i>Absent</i>	Black
Pale	White	Yellow	Pale	Chestnut-yellow
Black	Black	Black	Black	Black
Chestnut	Chestnut	Chestnut	Chestnut	Chestnut
—	—	—	Black	Black
—	—	—	Pale	Pale
—	—	—	Black	Black
Pl. XI, 1 B	Pl. XIV, fig. 10	Pl. XIV, fig. 12	Pl. XIV, fig. 15	Pl. XIV, fig. 8

The grey and chestnut areas at the base have been included in the table but their homologies are not clearly understood; possibly they are in some way connected with the barring, *e.g.* the chestnut may have formed a complete ring with the apical chestnut, the whole pattern being a modified eye-spot. Of this nothing definite can be said.

(c) *Wing coverts and remiges.*

These are all patterned feathers, but to describe intelligibly the somewhat complicated arrangement of these patterns would be difficult, and their bearing upon the more general problems has not yet been elucidated. Suffice it to say that a continuous series exists, passing down the inner covert feathers and then outwards through the secondary to the primary flights.

JUVENILE SPECIMEN (4) of *A. rufa*, bright variety
(Lord Deerhurst's bird).

This bird, which is very small¹, though superficially resembling the other bright birds, differs from them in possessing a number of feathers of the first plumage, especially on the head and wings.

¹ For example the tarso-metatarsal is 32 mm. and the middle toe (without nail) 28 mm. against 41 mm. and 38 mm. in adult. An excellent account of the various plumages of *A. rufa* at different ages is given by L. Bureau, "L'Age des Perdrix," *Bull. Soc. Sci. Nat. Ouest de la France*, 3^e Sér. 1913. I.

On the head, *black feathers have not yet appeared*—the small chick's feathers still persisting. Equally, the patterned feathers of the neck are absent, the throat being entirely clothed in greyish juvenile feathers. The breast has a mixture of grey feathers of the second plumage and pale chestnut feathers of the first. The feathers of the second plumage still retain their sheaths at their bases.

The flanks have the chestnut tips poorly developed, as is usual in young birds. One of these flank feathers shows very slight indications of a black bar outside the chestnut, followed by a further white fringe which is interesting since the true doubling of the distal black bar of the flanks is one of the unusual features of the corresponding variety of *A. saxatilis*, but is not found in the adult bright *A. rufa*.

In other respects the ventral side of the young bird is normal.

Dorsally, at a first glance, this bird is very different from the other bright birds. This is largely due, as were the abnormalities of the ventral side, to the presence of feathers of both first and second plumages.

Of the median dorsal feathers, those which are still in their sheaths agree closely with the feathers of the other bright birds. Those which are fully developed and about to be shed are almost devoid of barring, being pale chestnut with darker chestnut tips.

The scapulars and wing coverts include the same mixture of new and old feathers. The old feathers are of great interest since they agree more closely with the patterns of second plumage feathers of bright *A. saxatilis*. For example, many of these feathers have a heavy black bar on the outside while on the inside there are two bars separated by chestnut. The remainder of the feather is pale brownish yellow. Comparison with Figs. 8 and 9 in Plate XIV shows that these scapulars differ from those of bright *A. saxatilis* only in the loss of the proximal black bar, a difference which we should expect from the character of the normal flank feathers of the two parent species.

Between the anterior scapulars of the juvenile and adult bright *A. rufa* there is a much greater difference, namely, the true doubling of the distal black bar which has taken place in the first plumage only.

In the wing coverts there are similar points. Especially striking are the coverts which are next to the flight feathers. In the other bright *A. rufa* these have a black outer side and less developed black on the inside. The inside of each feather is covered by the outer web of its neighbour, so that in Plate XI these feathers show as a series of black lines. In the first plumage these feathers are black on the *inside* and pale on the *outside*, so that no black is visible externally when the

feathers are in place. In the bright variety of *A. saxatilis* the corresponding feathers are black inside with some irregular black outside. In this point, as in the doubled bar on the flank feather and on the scapulars, the first plumage of bright *A. rufa* resembles the later plumage of the bright variety of the other species, *saxatilis*.

Alectoris graeca saxatilis. "Bright" variety (var. *melanocephala* Fatio).

The general features of this form were described by Fatio, who applied to it the name var. *melanocephala*. In his paper there is a plate of this bird in black and white and coloured diagrams of several feathers.

In the light of what we know of the varieties of *A. rufa* it was very important to examine afresh the material which Fatio had described and to find out whether similar specimens of *melanocephala* had turned up since his description was published. As far as I know this has not occurred, but at Geneva there is a bird which will be described later since it shows some of the characters of var. *melanocephala* in a slight degree.

By the courtesy of the curators I was allowed to handle the specimens and to take several feathers from the Bex bird, of which some are figured in Plate XIV.

In this examination of abnormal *A. saxatilis* I set out to discover whether the variety was truly comparable with our bright *A. rufa*, i.e. whether precisely the same changes had taken place in the formation of the two varieties. Also I watched the seriation of the patterns of the feathers in order to arrive at some general idea as to the sort of changes likely to be found in series of feathers, the patterns of which are meristic. In this paper I shall describe such details of the seriation of patterns as bear upon the first problem of the relations between the two varieties, mentioning in passing a curious case of pattern-reversal. At present it is not worth while to enumerate the many un-correlated facts of feather decoration.

To explain the differences between the varieties it is necessary to bear in mind the differences between the parent species:

(1) The general body colour of *A. rufa* is reddish brown while that of *A. saxatilis* is grey.

(2) The striped flank feathers of *A. rufa* have only a single bar while those of *A. saxatilis* have two such bars (Plate XIV, figs. 5 and 10). As has already been mentioned this difference consists in the dropping out—in *rufa*—of the proximal bar which nevertheless occasionally reappears on the extreme anterior flank feathers (Plate XIV, figs. 13 and 14).

(3) The head and neck are very different in the two species. In each the throat is white and posterior to the throat is a black band, which curves down from the ears and is continuous mid-ventrally. Behind this in *A. rufa* there are many patterned feathers; these are absent from *A. saxatilis*, where the collar band is a very typical simple case illustrating the difference between *banding* and *barring*. At the anterior edge of the band in the median ventral line, the feathers are white at the base with a little black fringe at their tips. Behind these are feathers with rather more black. So, gradually, the black widens from a narrow fringe until it covers the whole feather, the white disappearing basipetally. This arrangement is modified in the var. *melanocephala*.

Description of *A. saxatilis* var. *melanocephala* Fatio.

The general coloration of both specimens of this variety is very like that of the bright varieties of *A. rufa*. The head is black, the gorget is reduced, the back and wings are striped. But in the details there are several anomalous points.

The throat is white (*i.e.* normal) but the gorget shows the following seriations in the mid-ventral line:—the first feathers have a greyish black fringe; in the following feathers there is a dark grey fringe and a dark grey base, leaving between the fringe and the base a paler bar. In feathers behind these, the fringe first fades away *in place*, then the dark base disappears basipetally. In this region, where there was no barring in the normal, a form of barring has been developed resembling slightly that seen on the corresponding feathers of bright *A. rufa*, but differing markedly from the barring on the neck of normal *A. rufa*.

The flank feathers are very abnormal in the var. *melanocephala* (Plate XIV, fig. 7). The chestnut tip is much reduced—a juvenile characteristic present on the two specimens, one of which is adult. The two black bars have become very irregular and smudgy and in some cases fuse together in the centre of the feather. Many of these feathers show a true doubling of the distal bar. In Plate XIV, fig. 7, this is very poorly shown, but careful scrutiny reveals a paler, narrower triangle invading this distal bar from the left-hand side. This triangle is much clearer on the actual feather, especially when seen against a black background.

In the feathers of the lower breast and abdomen is another important difference between the bright variety of *A. saxatilis* and that of *A. rufa* where they remained normal. These belly feathers are in continuous

series with the striped flank feathers. The barring has descended on to them so that they all show black lines on an otherwise self-coloured ground. On the more anterior belly feathers there are two such lines corresponding to the two lines on the flanks, but the feathers are somewhat asymmetrical, the distal band being most strongly developed on the outer side. This asymmetry is what we should expect from the character of the normal flank where the more ventral feathers have the bars less formed on the ventral (or inner) side.

Posterior feathers of the belly have no distal bar which faded away in place, but the proximal bar remains on the yellow chestnut ground. The symmetry has undergone an unexpected change, the bar being best developed on the median side of the feathers. Traces of the black bar are on all ventral feathers, even the small feathers immediately in front of the anus having blackish smudges.

Dorsally this variety agrees with the bright form of *A. rufa*, as has been stated, and still more exactly with the first plumage of that variety. Among the median dorsal group of feathers those of the mantle have the proximal bar characteristic of the species poorly developed. The distal bar has partially undergone true doubling; so that on each side there is a horseshoe-shaped black mark filled up by chestnut which has come in from the outer side. On the more lateral feathers of the group—*e.g.* feathers from the same region as Figs. 3 and 12, Plate XIV, which are from *A. rufa*—the distal bar is single on the outside and very broad, while on the inside web the true doubling persists. Feathers of this type are common on the back of this variety, as they are on that of the juvenile bright *A. rufa* in its first plumage.

Posteriorly in the median dorsal group the old proximal bar disappears in place, the distal bar persists very broad on both sides of the feather and frequently shows its double nature by partially dividing, leaving chestnut between the two bars so formed, as it did in the first and later plumages of the bright *A. rufa*. This bar gradually fades away in place, giving a complete seriation of colours down the back to the still normal rectrices.

The scapular feathers (Plate XIV, figs. 8 and 9) have the proximal bar well developed, but asymmetrical. Fig. 8 shows a feather of a common type from the left side of the bird. On the outside web is a single black bar of doubtful homology, probably a fusion of proximal and distal bars, judging from other similar feathers on which this external bar is better developed. The grey reaches clearly up to it and there is a slight invasion of the bar from the rachis by a paler triangular area.

On the inside web there are three bars, viz. a true proximal bar and a doubled distal bar.

Among these patterns there occurs a reversal of symmetry quite unexpectedly. Plate XIV, fig. 9, represents one of these reversed patterns. The feather from which the figure is taken lay immediately under that shown on Fig. 8. It will be seen that the outside web of Fig. 9 is a very fair mirror image of the inside of Fig. 8. But on the inside of Fig. 9 the copying is not so exact and, instead of the single bar, which was described above as a fusion of the proximal and distal bars, we get two bars, the proximal having shifted outwards towards the distal bar without fusing with it.

This reversal seems to us very remarkable. It is present on both sides of each of the two specimens of the var. *melanocephala* but it only affects a very small number of feathers in each case—three at most. There are occasional feathers of intermediate symmetry, of which we have one on which there is a proximal bar on the same part of the web on each side, while the distal bar is symmetrically doubled. The feathers on *A. rufa* corresponding to these reversed feathers show but little change of the same kind; the chevron mark is perhaps somewhat altered, but initially it was so nearly symmetrical that no conclusions can be drawn from this. No hypothesis can at present be put forward to explain the reversal beyond saying that the scapular pterylosis perhaps constitutes an *independent* system of symmetry—independent of the more general controlling mechanism which lies behind the bilateral symmetry of the body as a whole. This emancipation of the scapular pterylosis is perhaps correlated with the increase of barring since it is possible that the latter is an effect of some change whereby the feathers have been allowed to act as individuals with their own pattern-control, independent of the more general system which lies behind the *banding*.

The wing coverts need not be described at length apart from mentioning again the symmetry of the coverts which overlie the flight feathers—one of the points of agreement between this variety and the first plumage of the bright *A. rufa*. The seriation of patterns follows much the same lines as in *A. rufa*.

The secondary remiges are pale feathers with black tips and irregular blotches of black on the centre along the rachis. This black increases from feather to feather as we go outwards until on the outer primaries there is only a very little pale colour in broken patches on the inside of the feather. The primaries have no chestnut on the basal region of the

outer vane to correspond with the chestnut present on the adult bright *A. rufa*. This chestnut is absent too from the first plumage.

THE GENEVA SPECIMEN. (Partially "bright" *A. saxatilis*, No. 3.)

When on my way to Bex and Sion to see Fatio's birds, I stopped at Geneva and visited the University Museum to look for any variation which might bear upon the two aberrations. I found there one very interesting specimen of *A. saxatilis*, a female, which had a few abnormal striped feathers on the back, and in a small degree some of the other characters of Fatio's birds. I made a description of this bird before I had seen the two bright specimens at Bex and Sion, so that the description here reproduced was not affected by a knowledge of the details of those birds.

Description. The general colouring and size are normal. The head and throat are normal, but the black gorget is somewhat reduced ventrally. This is quite marked: there are fewer black feathers and less black on them. The breast is normal. The pale brown belly is normal.

The barring of the flanks extends *further ventrally than usual*. This may have been due to the method of stuffing, but I considered this possibility at the time and made a note saying that I did not believe that the abnormal appearance was due to this. The skin has been sewn up in such a way that the mid-ventral seam is pushed inwards, a distortion which might cause the flank feathers to appear to extend towards the mid-ventral line. The symmetry of the flank feathers is normal. The rectrices are normal.

On the back there are seven abnormal feathers which I described as being all in the scapular pterylosis, though when I took my notes I was not fully able to separate mantle feathers from those of the scapular region. It appears from the curvature of the specimen in Plate XIV, fig. 11, that the feather belongs to the mantle.

On the right side there were three barred feathers inserted close together in what I then regarded as the posterior part of the scapular pterylosis. They all had the same type of pattern and asymmetry which were best developed on the posterior of the three feathers and least clear on the middle feather. (The posterior feather is reproduced in Plate XIV, fig. 11, thanks to the courtesy of M. Bedot, the Curator of the Museum.) The aftershafts of these three feathers were paler than those of their neighbours.

On the left side there were four abnormal feathers, three of which were inserted close together, forming a small patch somewhat posterior

to the corresponding patch on the other side of the back. The patterns and symmetry were substantially similar, the feathers on the left being mirror images of those on the right. The bars were much more indefinitely formed. The fourth feather on the left side was much further forward, having its tip near the angle of the wing.

There were no other abnormalities in the colour of this bird, except a few irregular spots of black on one or two of the mantle feathers.

Thus the bird has the following points in common with the var. *melanocephala*:

- (1) Seven barred dorsal feathers.
- (2) Some ventral extension of the barring usually confined to the flank feathers.
- (3) Some reduction of the collar band.

G. B.

DISCUSSION.

Nature of the Aberrations.

On this head we have little to remark. Nothing suggests that either aberration is a product of hybridisation and that hypothesis may be dismissed. Those who are interested in the evidence as to alleged hybrids of *Alectoris* or of *Perdix* will find a full collection of records and discussion in Suchetet's most valuable treatise¹. Amongst various suspicious cases Fatio's specimens are considered by Suchetet and we are satisfied that readers will agree with him that the "bright" variety of *A. saxatilis* cannot have resulted from hybridisation with any other species², though with doubt Suchetet admits that certain other specimens may have been true hybrids between *rufa* and *saxatilis*. Our two aberrations of *A. rufa* were not known to him. To Suchetet's discussion we may add that not only is a cross with some other species of Partridge ruled out, but, apart from several other considerations, the absence of the slightest structural departure from the normal disposes of any idea—in any case very remote—that crosses with domestic poultry can have produced these results.

No opportunity has yet occurred of making a histological examination of the genitalia of these aberrations. Though such examination is desirable we see no reason to anticipate that any form of pathological

¹ A. Suchetet, *Des Hybrides à l'état sauvage*, Lille, 1896, Vol. I, *Classes des Oiseaux*, pp. 6-10 and 485-499.

² Incidentally he mentions, pp. 9 and 487, a bird in the Museum at Marseilles as possibly a further specimen of Fatio's var. *melanocephala*.

sterility is responsible for either. In both *rufa* and *saxatilis* the sexes normally differ very little, if at all, in plumage. Externally, apart from the presence or absence of spurs they cannot be certainly distinguished from each other and it is therefore almost out of the question that either aberration, still less both, can be a consequence of sexual derangement. It is to be remembered that the three dull *rufa* were males, and at least two of the bright *rufa* are also males. The third bright *rufa*, though the sex is not recorded, is almost certainly ♀, since though a full grown bird no spurs are developed. One of the bright *saxatilis* is ♀, and the Geneva *saxatilis* which had some "bright" features was also ♀. As regards the bright aberration therefore we have proof that it may manifest itself in either sex¹.

We look on the two aberrations as due to changes in the factorial composition of the normal, proceeding in opposite directions, but as to the physiological cause of these changes we have no suggestion to offer.

The Nature of the Factorial Changes.

No attempt to bring the several forms into one consistent scheme of factorial representation has been successful. In our present ignorance of the mechanical and chemical processes by which pigments are distributed over the surface of the body such hopes can be scarcely entertained. Nevertheless, on comparing the dull, the normal and the bright birds together, their distinctions may evidently in part be represented as a series of terms in the development of colour-segmentation. In the dull bird no segmentation or barring of colour occurs, whether on single feathers or as affecting groups of feathers except on the neck. In the normal, barring has occurred on the feathers of the flank, and in the bright birds this process is extended over the mantle and scapulars and in bright *saxatilis* over the belly also.

For such a series of terms in the progressive spread of barring analogies may be found in the banding of the Equidae. In the various species of Asses stripes may be absent or only slightly developed on the extremities. In the extinct Quagga (*Equus quagga*) the striping was complete on the head and was continued backwards over the withers and thorax, dying out in the middle of the body, leaving the hind quarters and legs self-colour. In the Zebras this striping is complete over the whole body and legs, the light-coloured belly alone excepted.

¹ It is perhaps worth noting that probably none of these females were sexed by their genitalia and that we can only guess at the effects of pathological sterility upon the spurs, but since the Norton Hall bright bird has very large spurs, and the Kinver bird though adult has none, sexual disturbance cannot be suspected as the source of the aberration.

But the other changes in the partridges appear to be independent of the spread of barring.

Nevertheless the various distributions of the colours in the rectrices and tail coverts obviously form a progressive series of three successive terms of which the normal is again the middle. In the dull birds the whole tail with its coverts is approximately of one colour. In the normal the rectrices are chestnut with the coverts unaffected. In the bright birds the chestnut of the rectrices has spread up to include the tail coverts.

If these were the only distinctions the three terms would naturally be regarded as ordinary progressive distinctions, which, if referable to genetic factors, would on analysis presumably fall into a multiple allelomorphous series; and though making no suggestion as to which end of the series should be regarded as positive and which negative, we incline to accept this interpretation. When, however, we come to the characters of the head and throat we are at a loss. The three arrangements appear to be entirely independent of each other, nor can we suggest any way in which any one can be readily interpreted as a derivative of any other. The greatest difficulty arises in connexion with the black head of the bright variety, which is probably a definite accompaniment of that variety in both the species concerned, for, as stated above, we incline to interpret the head of Lord Deerhurst's bird as differing in juvenility only. We have to remember that a form exists in south-west Arabia which has a black head, the species *melanocephala* Rüpp. This is evidently a distinct species, characterised by great size and certain peculiarities of coloration, amongst which a black head is the most striking feature. This black head may no doubt be factorially of the same nature as that in the bright varieties of *rufa* and *saxatilis*, but nothing else in the plumage of this species *melanocephala* is suggestive of the other distinguishing characteristics of our bright birds.

If the features of the throat, stood alone we might perhaps incline to conjecture that the dingy throat and weakly marked gorget of the bright birds meant that the normally sharp differentiation into white and black was suppressed, just as the waves of differentiation die out on the Quagga. On another line of interpretation the black throat of the dull birds might be supposed to be the black of the normal gorget which had not been pushed back into its typical position. But there is little to commend such suggestions, and we see no scheme by which analysis of the distinguishing features of the head, throat and breast can be attempted.

Very similar and equally insoluble difficulties arise in regard to the features distinguishing the head and neck in the varieties of *Colaptes* (the American Flickers. PICIDAE) known as *auratus*, *cafer* and *chrysoides*¹. Here again we have varieties in which certain distinguishing features cannot be represented as in any sense readily transformable into each other. For example, in *auratus* the throat and foreneck are brown, and the top of the head and the hindneck are grey, but in *cafer* the colours are exactly reversed, the dorsal parts of head and neck being brown and the ventral part grey. Moreover the development of the other distinguishing marks, especially the nuchal crescent and the colour of the malar stripes, cannot be readily ascribed to the operation of a single factorial element. Nor have we any indication to guide us in the attempt to decide which of the varieties is due to a factorial gain and which to a factorial loss.

Besides the species, *A. melanocephala*, mentioned above, there is another related species which may throw some light on the increase of barring, namely, *A. petrosa*, in which the scapulars are normally somewhat patterned. The feathers have bluish-grey centres and bright chestnut margins. The chestnut is sharply separated from the grey but there are no black bars. Extreme anterior flank feathers of normal *A. rufa* have somewhat this appearance so that perhaps in *A. petrosa* the barring has normally an influence on the scapular feathers.

In attempting to form a conception of the way in which rhythmical banding develops and spreads over the body of an organism, or rhythmical barring over an organ such as a feather, the analogy with the propagation of wave-motion must in part, at least, be a true guide². The terminology is immediately applicable. As Whiting, for example, has done in a discussion of the banding of Cats' skins, we may in such cases perhaps speak of these bands as "waves of pigment-forming metabolic activity"³, and the terms "crest" and "trough" which he has used in reference to the banding of a moth's wing⁴ are probably more than merely metaphorical expressions. We may even go further, and applying the analogy of wave-length we may speak of the pattern on Grévy's Zebra (*Equus grévyi*) as approximately the upper octave of that of *Equus zebra*. Such a terminology by familiarising the mind with the

¹ For an account of these see W. Bateson, *Problems of Genetics*, Newhaven, 1913, pp. 147-156, coloured plate.

² See especially *Problems of Genetics*, 1912, Chap. III.

³ P. W. Whiting, *Journ. Exp. Zool.* 1918, xxv, p. 551.

⁴ *Ibid.* 1919, xxviii, p. 440.

probable nature of the process of these segmentations will prepare the way for a correct analysis, though admittedly open to abuse.

Attention is finally called to the curious development of a minor system of symmetry in each scapular region in the bright varieties, as indicated by the occurrence of feathers within one pterylosis which are partially mirror-images of each other.

In the course of our inquiries we have had help from many ornithologists. We feel under a special obligation to Mr P. R. Lowe for entrusting us with this curious and valuable material. Our thanks are due both to him and to Mr N. B. Kinnear of the British Museum for their continued help in a field with which we have little familiarity.

For assistance in connexion with the study of the specimens in Swiss Museums we must express our gratitude to M. Paquier of Bex, who gave special facilities, to M. C. Meckert of Sion and to MM. Bedot and Revillod of the Geneva Museum.

NOTE. After this paper had been written we learnt through the kindness of Mr Lowe of two more specimens of the "bright" variety of *A. rufa* killed in Spain. He sent us a paper¹ by Ignasi de Sagarra in which the second of these, found near Valencia, was described with figures and a good coloured plate. The bird agreed in all respects with our var. *melanocephala*, and it apparently resembled the Overbury bird in having a nuchal band. Sagarra refers to two papers by Soler² dealing with the earlier specimen, the locality for which is given as "Sant Llorenç del Munt" in the extreme east of Spain.

Both birds had some greyish feathers scattered through the black cap which Sagarra regards as transitory and possible indications of changing plumage.

Both authors consider these birds as belonging to a new species. In his first paper² Soler called it *Perdix melanocephala*, but in a further note in the same Journal³ he substituted the name *Perdix ornata*, since *melanocephala* was already in use for the Arabian species.

We cannot find the particular San Lorenzo, but Sagarra's remarks indicate that (p. 9) it must be more than 200 miles from the Valencia locality.

¹ I. de Sagarra, *Noves Ornithòlogiques, Treballs de L'Inst. Catalana d'Hist. Nat.* 1915, p. 5.

² *Bulleti Inst. Cat. d'Hist. Nat.* 1904, p. 51, with a photograph.

³ *Ibid.* 1906, p. 16.

Soler's bird was found among normals. Sportsmen in the previous year had, however, mentioned seeing similar birds which they had called "*Perdius canaris*."

Finally, as the sheets were passing through the press, we have found a letter by A. Vaucher (*Boll. Soc. Ticinese Sci. Nat.*, 1913, VIII, p. 106) addressed to Ghidini giving various details as to the variability of *saxatilis*. For the most part these appear to have no direct relation to our problems, but M. Ghidini appends this interesting note, "J'ai observé souvent sur le croupion des bartavelles, des plumes qui montraient une coloration se rapprochant de celles des flancs, presque toujours par groupe de trois: p. ex.: une femelle du Val Bavona du 31. I. 1911." Evidently the reference is to such specimens as the Geneva bird from Osières, which had been presented by M. Ghidini himself. These *saxatilis* with chevron feathers on some part of the back "*par groupe de trois*" may be regarded as another term in the series between the normal and the var. *melanocephala*.

DESCRIPTION OF PLATES XI—XIV.

PLATES XI and XII. *Alectoris rufa*. In each plate, A is var. *obliterata*, the Braintree specimen of the "dull" variety. B is the normal. C is the var. *melanocephala*, the Norton Hall specimen of the "bright" variety.

PLATE XIII. Individual feathers taken from positions, approximately corresponding, on the left side in the three forms. A, B, C, the three forms as before, but the feathers are not in each case all from one specimen.

- | | | |
|----------------------------------|---------------------|-----------------------------|
| Fig. 1. Flank. | Fig. 2. Breast. | Fig. 3. Median wing covert. |
| Fig. 4. Anterior part of mantle. | Fig. 5. Back. | Fig. 6. Rump. |
| Fig. 7. Belly. | Fig. 8. Lower neck. | |

PLATE XIV. Figs. 1-6 *Alectoris rufa*.

Figs 1-3 are left mantle.

- | | | |
|-----------------------|-----------------|-------------------------|
| Fig. 1. Dull variety. | Fig. 2. Normal. | Fig. 3. Bright variety. |
|-----------------------|-----------------|-------------------------|

Figs. 4-6 are from the right flank.

- | | | |
|-----------------------|-----------------|-------------------------|
| Fig. 4. Dull variety. | Fig. 5. Normal. | Fig. 6. Bright variety. |
|-----------------------|-----------------|-------------------------|

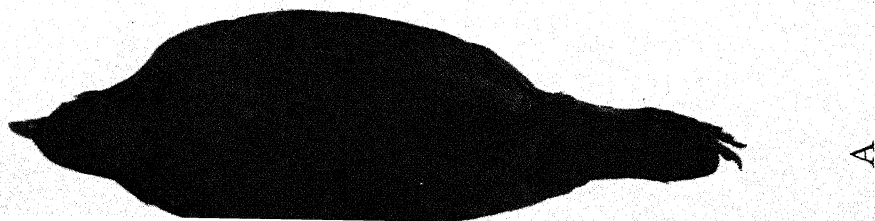
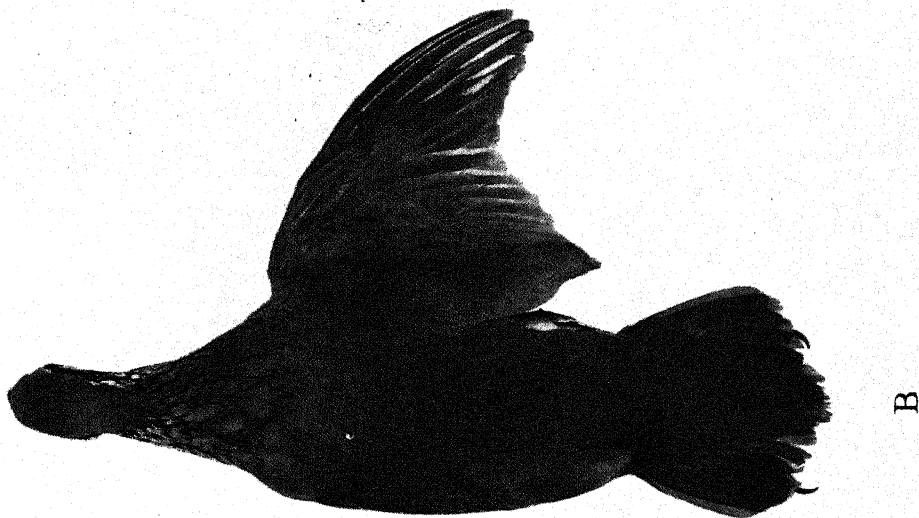
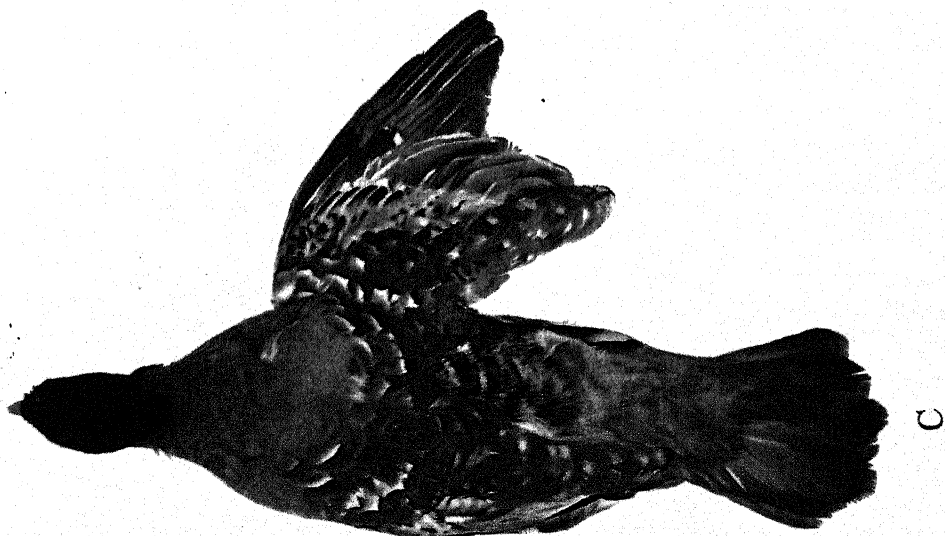
Figs. 7-11 *Alectoris saxatilis*.

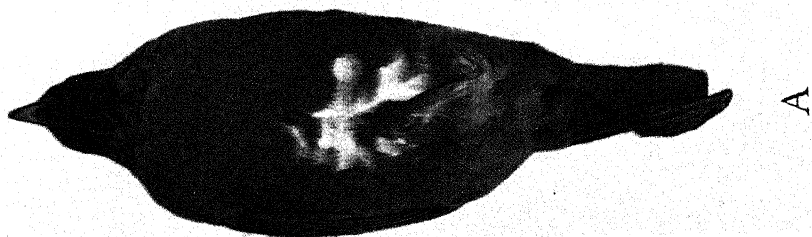
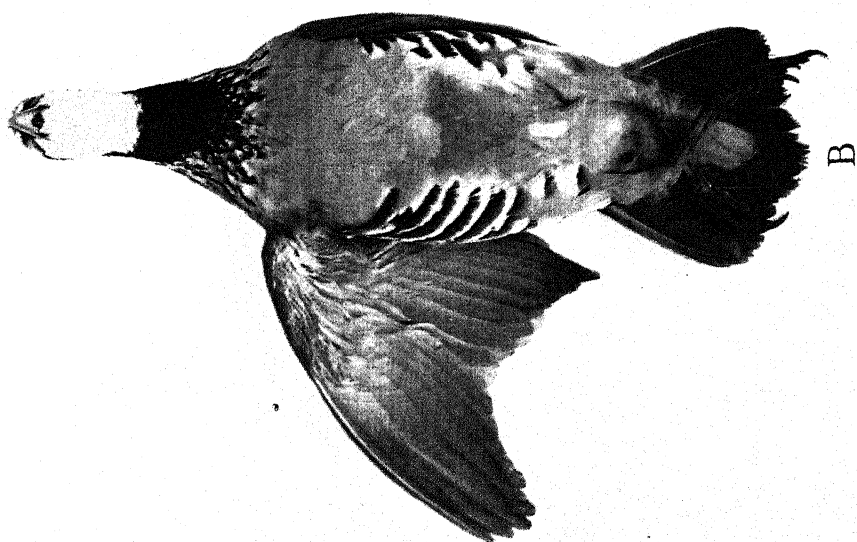
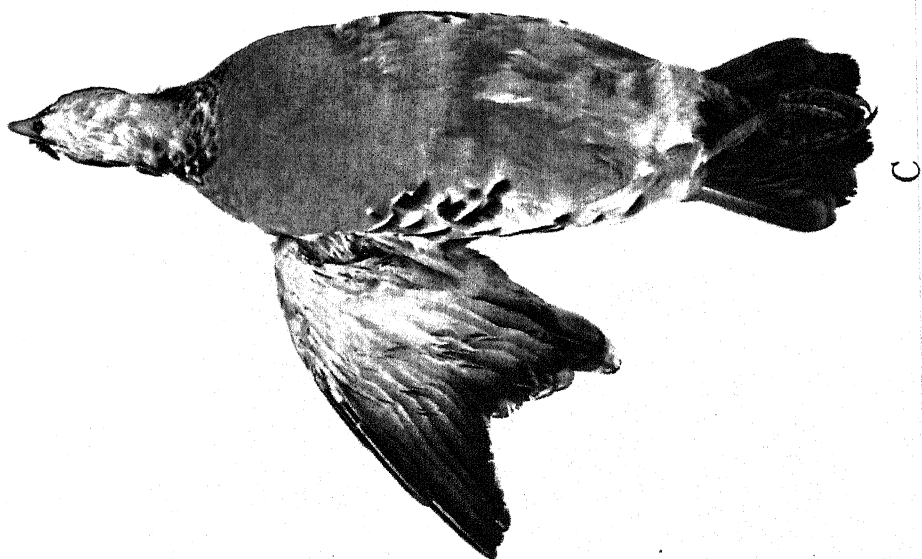
Fig. 7. Var. *melanocephala* Fatio, the Bex bird. Right median flank feather, showing some chestnut invading the black bar.

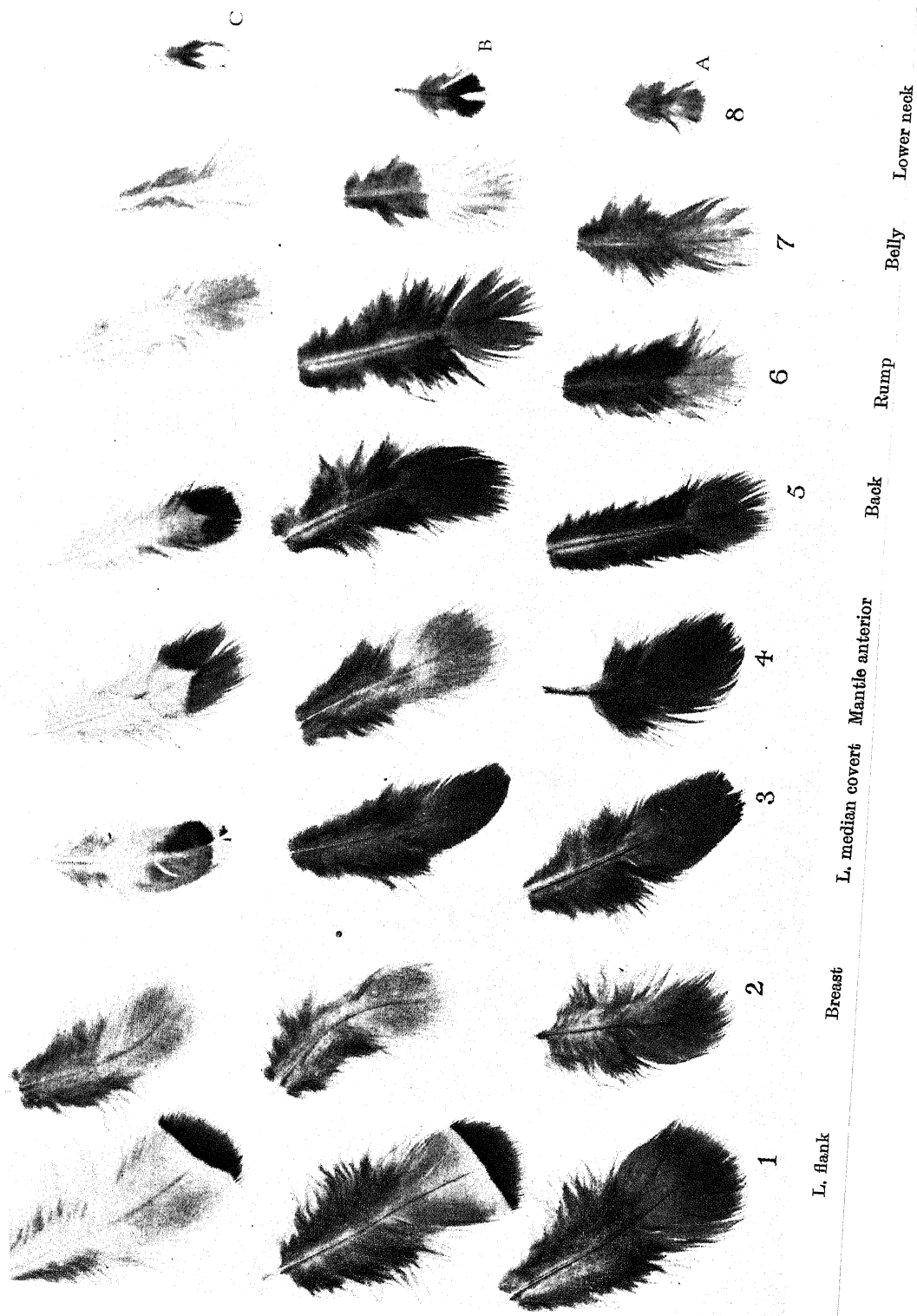
Fig. 8. Var. *melanocephala* Fatio, the Bex bird. Left posterior scapular of the usual pattern, with ashy blue area on the external web, and three dark bars on internal web.

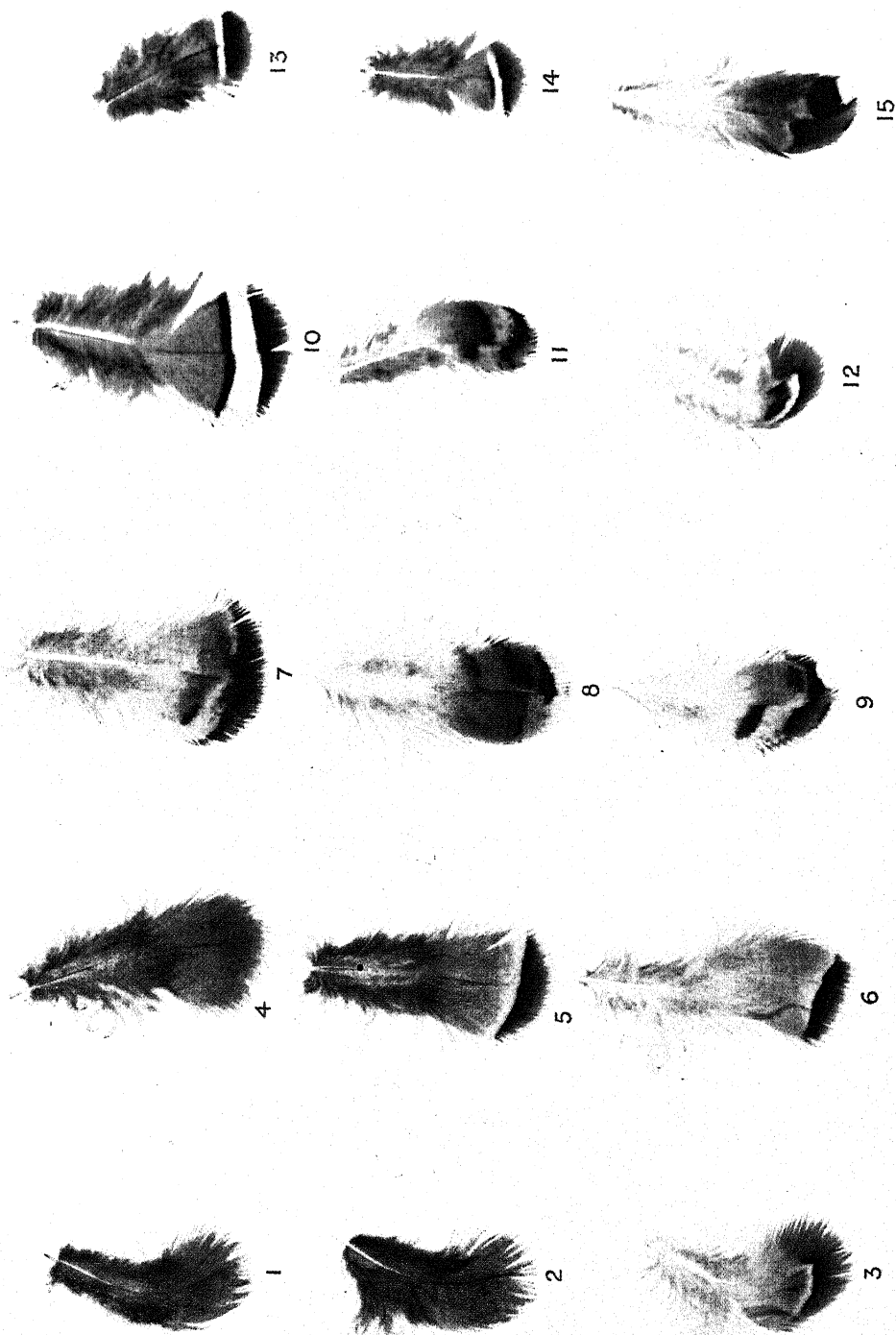
Fig. 9. Var. *melanocephala* Fatio, the Bex bird. The exceptional or "reversed" pattern with trace of ashy blue area on internal web (scarcely visible in the colour print) and three dark bars on the external web.

Fig. 10. Normal flank feather (left median).









- Fig. 11. Right scapular (or mantle?) from the Geneva specimen of *A. saxatilis* showing the pattern peculiar to var. *melanocephala* Fatio. One of three such feathers in this position.
- Fig. 12. A left mantle feather of *A. rufa*, var. *melanocephala*. British Museum mounted specimen Reg. No. 1904.10.2.1, showing the proximal bar exceptionally well developed. Compare Fig. 3 on same plate which shows the usual appearance of such feathers in this variety.
- Fig. 13. *A. rufa*, normal. Right anterior flank feather showing the proximal black bar on external web.
- Fig. 14. *A. rufa*, normal *juv.* Right anterior flank. Proximal black bar on both webs, as in normal *saxatilis*. Such feathers are uncommon in *A. rufa*, and occur only at the anterior end of the scapular series.
- Fig. 15. *A. rufa*, var. *melanocephala*: left posterior scapular, showing doubling of the distal black bar.

HEREDITARY PREDISPOSITIONS TO DIZYGOTIC TWIN-BIRTHS IN NORWEGIAN PEASANT FAMILIES.

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(With Thirteen Text-figures, Nine Tables and Three Plates.)

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CHAPTER I.

INTRODUCTION.

(a) *Historical Notes.*

Our present knowledge with regard to the occurrence and nature of multiple births in man, has been reached during the last century through investigations following three main lines, *statistical*, *clinical* and *genealogical* respectively.

(1) The statistical investigations of multiple births are chiefly based on the great statistics of Veit (1855) for Prussia and of Wappäus (1859) for a whole series of European countries, each of these statistics covering many millions of births. The general frequency of multiple—or especially of twin-births was here at once established as amounting to about 1 in 80

¹ The new name of Kristiania.

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births, representing a percentage of the total number of births varying between 0.93 and 1.5. The sexual ratio for twins was shown to be very nearly the same as for single births.

A large number of later statistics from various countries or districts, have served to support these results of Veit and Wappäus.

An interesting investigation was made by Duncan (1865 *a*), regarding twin-births in relation to the age of the mothers. As a result he was able (p. 772), "to state the law that from the earliest childbearing period till the age of 40 is reached, that is, till a period when fecundity has become extraordinarily diminished, the fertility of mothers in twins gradually increases." He also raised the question about a causal connection between twin-births and the number of pregnancies, a question which in a following paper (1865 *b*), was answered by him in the affirmative, "the increased frequency of twin-bearing as the number of the pregnancy increases" being here considered "as a law of the production of twins" (p. 929). The relation of twin-births, finally, to the general fertility of the twin-mothers was left by him as a question to which the material at his disposal could give no definite answer.

Upon this point his work was continued by Puech (1874), who after a very thorough statistical review of the occurrence of multiple births in the various European countries states the following results (p. 239): "...la fécondité et l'aptitude aux grossesses multiples sont deux caractères contingents, variant d'individu à individu, de contrée à contrée, et de peuple à peuple, présentant l'une et l'autre des oscillations en parfaite concordance, mais étant subordonnées l'une à l'autre.

En définitive, le degré de fécondité des femmes est la loi, d'après laquelle se repartissent les grossesses multiples. Quant aux autres agents, ils n'ont d'effet que par leur mode d'action sur celle-ci."

The same question was investigated and discussed also by Prinzing (1907), who supports the view of local differences with regard to twin-production without, however, considering the fertility of the race as a sufficient explanation.

With regard to *triplets* statistical investigations corresponding to those above mentioned have been made by Mirabeau (1894) and by Saniter (1901), while the occurrence of *quadruples* was made the subject of investigation by Brattström (1914).

(2) Parallel to and amplifying these statistical investigations, clinical evidence with regard to multiple births has been equally developed especially through the investigations of Spaeth (1860), Ahlfeld (1875), Strassmann (1889-1908), Rumpe (1891).

Of special interest are here the results reached with regard to the placenta and the secundines of these births, to the effect that the number of chorions should be considered decisive for the nature of the multiple birth. Two originally separate placentas may often fuse to one, and with very few exceptions each one of the embryos, whether they develop from one or from two or more eggs, are covered by a separate amnion; but all investigators unanimously maintain that the number of eggs developing may be determined by the presence of a corresponding number of chorions. A further result, equally beyond dispute is that twins (or triplets, etc.) contained within one and the same chorion, that is monozygotic twins, are found to be always of the same sex, while in the dizygotic twin-births, pairs of different sex are also found.

Rumpe (1891), in his statistics upon clinically investigated material, has found that dizygotic twins are born (p. 346) "vorwiegend von Müttern im vorgeschrittenen Alter (über 25 Jahre)," while monozygotic twins are found to be "in jedem Geschlechtsalter gleich oft beobachtet." The sexual proportions of mono- and of dizygotic twins separately, stated by Spaeth (1860), Strassmann (1889), Rumpe (1891), Brém (1891), Quenzel (1898), Schapiro (1912), Neuhauser (1913), Meyer (1916) will, as well as the papers of Weinberg (1902-1912), be considered in a following chapter¹.

With regard to the etiology of multiple births the authors seem to agree in looking upon the monozygotic group as due to some unknown anomaly in the early development of the egg (see especially Stockard 1921).

For the dizygotic twin-births the basis has been sought above all in the ovary of the twin-mother, the multiple birth being considered as caused either by a simultaneous rupture of two Graafian follicles, or by the existence in one follicle of more than one egg. Investigations of ovaries have proved that, as an anomaly, a structural basis may be found for a bursting of two or more follicles at a time (Hellin 1895, Patellani 1896, Olshausen u. Veit 1899), as well as for two or more eggs being contained in one and the same follicles (Schrön 1863, v. Franqué 1898, v. Bumm 1902, Arnold 1912). In both cases the ovaries have been characterised also by the maintainance up to the adult age of an abundance of primordial follicles, thus giving a picture which is normally found only in young individuals. Such ovaries have been described as

¹ The very significant book of T. Wedervang on *Sex Proportion and its Variations in Relation to Ante-Natal Mortality*, Oslo, 1924, has appeared too late to be considered in this paper.

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being similar to those of multiparous animals, and the relatively frequent occurrence in man of multiple births has been taken as a sign, that the uniparity has not yet been fully established (Hellin, Strassmann a.o.). Strassmann (1908) draws our attention to the fact that in cases of an *uterus bicornis* which, as is well known, may be found as an anomaly in man, twin-births (Dunning 1889) occur in a proportion of 1 in 18 births, instead of the usual frequency of 1 : 80.

While such ovarian predisposition to twin-births generally has been considered as an anomaly, the opinion has on the other hand been maintained by Neugebaur (1913) and quite recently also by Davenport (1920), that a simultaneous ovulation of two or more eggs in reality is a more common phenomenon than is generally admitted, one of the two eggs being either not fertilised or at some early stage checked in its development. "Oft genug machte ich die Erfahrung," says Neugebaur (p. 1063): "hätte ich die Placenta nicht untersucht, so wäre die Zwillingsschwangerschaft ganz übersehen worden."

It is of interest in this connection to glance at the corresponding relations in mammals, known to us especially through the papers of Käppeli (1908), Hammond (1914) and Küpfer (1920, 1923).

The domestic animals investigated all differ from each other with regard to the numerical relations between *corpora lutea* in the ovaries and embryos in the uterus. In the domestic swine the number of *Graafian* follicles burst during a heat-period is very high, average 16¹, the number of embryos varying around half this number (Küpfer 1920). In sheep, on the other hand, there is a more general correspondence between the two figures, even if in about 30 per cent. of all cases the number of *corp. lut.* may surpass the number of embryos by one, or in exceptional cases even by two or three (Küpfer 1923). In cattle, finally, the correspondence between the numbers of *corp. lut.* in the ovaries and embryos in the uterus is, normally, found to be absolute (Lillie 1917, Küpfer 1920, 1923).

The discrepancy between ovary and uterus, so striking in domestic swine, must according to the interesting investigations of Käppeli (1908) be looked upon as an effect of domestication itself, not only the fertility but the whole sexual cycle of domestic swine being widely different from those of wild swine. In wild swine a heat-period occurs once a year, in the autumn, and the number of young born is in average four, while in the domestic swine heat-periods return every three weeks and the number

¹ According to Hill and Donoghue (1914) the number of eggs simultaneously ovulated may in Marsupials (*Dasyurus*) amount even to 35.

of ripe follicles in the ovary in each period has been raised to an average of 16. No wonder, then, that there may occur some discrepancy between ovary and uterus with regard to the speed of their adaptation to this new mode of life. As demonstrated by Hammond (1914) atrophic foetuses are found in the uterus of the sow which are (p. 270) "not all of the same size but appear to have dropped out one by one in a manner such as would occur in a struggle for nourishment with the increasing bulk of the foetuses," while other cases seem to indicate that also "reduced vitality" may play a part in this atrophy of the embryos. Both phenomena would find a natural explanation as features following the adaptation to domestic life.

Similar, but less conspicuous, differences with regard to the heat periods have been shown (Käppeli, Küpfer) to exist also between the more natural and the highly cultivated races of sheep and cattle, while in goats as well as in dogs and cats the ovarian cycle has remained that of wild animals. The experiments of Käppeli have proved, however, that even in goats the ripening of ovarian follicles, and therefore also the occurrence of heat, may be accelerated through over-feeding.

An interesting fact, also revealed through the investigations of Käppeli, is that the number of primary follicles in the ovary is considerably reduced during domestication, while with the acceleration of their ripening the size as well as the weight of the ovary is very considerably augmented.

The transition from wild to the domesticated mode of life seems, therefore, to have a similar effect on the ovary as that demonstrated by von Hanseemann (1912) for the development of an individual to sexual ripeness, and characterised by him as "ein Kampf der Eier in den Ovarien." Each ripening Graafian follicle induces upon its surroundings an effect causing atretical degeneration of a whole series of other follicles.

Now, returning to the question raised by Neugebaur and Davenport (see above p. 128) with regard to the actual number of double ovulations in man, we may, no doubt, compare his sexual cycle with that of domesticated animals.

In the multiparous domesticated swine with its high degree of over-feeding, the number of young born was found to be considerably lower than that of follicles burst, and at the same time the size of each litter was found to have a wide range of variation, from 6 to 19 young (Käppeli 1908).

In sheep there was a much more stable relation between the number

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of ovulations and that of foetuses, as well as in regard to the number of young born, the sheep thus standing between the multiparous and uniparous animals in so far as it displays a very high percentage (24 per cent. [Marshall 1904], 36-60 per cent. [Bell 1904, 1912]) of twin- or triplet-births. The number of ovulations was found to surpass that of embryos in about 30 per cent. of all cases, while in the great majority of cases these numbers agreed.

In cattle the *uniparity* as well as the agreement between ovulations and embryos were shown to be very stable, twin-births occurring (Jones and Rouse 1920) in a percentage of 0.4 only, and scarcely any deviation having been found between the numbers of ovulations and embryos. In horses the percentage of twin-births will probably prove to be similar to that of cattle, although the groups of material so far investigated (see Robertson 1913) are too small to give reliable results.

With regard to *uniparity* man should, with its 1.2-1.4 per cent. twin-births, range near the cattle. If we may judge, therefore, from the stability here displayed in the correspondence between ovulations and embryos, it seems probable that we shall find such correspondence also in man. Such conclusion agrees indeed very well with a long series of investigations of the ovaries of pregnant women.

(3) Clinical investigators have in their communications given many examples of an individual, as well as of a hereditary disposition to multiple births, while other authors, especially Weinberg (1902-12), have treated the question as to heredity by an ingenious use of statistical material. But for a proper treatment, the application of genealogical investigation is manifestly required. Among the authors who have made genealogical study the main object of their work may be mentioned in the first place Goehlert (1897), v. Speyr (1894), Rosenfeld (1903), Oliver (1912), Jordan (1914) and Meyer (1916).

Some of the pedigrees given by these authors comprise a great number of individuals, belonging to a relatively large number of generations. This is the case especially with the pedigrees of princely families presented by Goehlert and by Meyer. But these pedigrees show, on the other hand, the very serious disadvantage that very often the female lines of the families have not been worked out. For hereditary questions, and especially for questions so complicated as that of the heredity of twin-births this deficiency will make the pedigrees more or less useless.

As a matter of fact no conclusion has been reached as yet with regard to the type of heredity of twin-births. A review of the most recent litera-

ture shows great disagreement to exist in the opinions, and even in the evidence, with regard to dominance or recessiveness, as well as to the question of the responsibility of each of the twin-parents in the heredity.

A consideration of the heredity of twin- (or multiple-) births will, however, at once reveal a series of difficulties which explain why no definite solution has yet been reached.

Among such difficulties should in the first place be mentioned:

(1) The double nature of twin-births, the mono- and dizygotic, representing genetically absolutely different phenomena, while phenotypically a distinction between both groups may in many cases prove quite impossible.

(2) The fact that hereditary disposition for twin-births will not prove its existence until in the next generation. All women without, or with only few, descendants should, therefore, be excluded from our considerations.

(3) The fact, further, that so far as dizygotic twin-births are concerned the phenotypical appearance of an hereditary disposition must, according to its nature, be *sex-limited*. No man can, even if carrying the twin-heredity as a homozygote, directly prove the existence of such disposition in the F_1 generation. In the following generations also the inheritance may, if conducted through males only, remain imperceptible to the investigator.

The difficulties here mentioned will, to anyone familiar with genetics, prove very serious—so serious indeed, that absolutely definite results as to the type of heredity may be unattainable. The many interesting questions connected with hereditary disposition to multiple births play, however, a considerable part in scientific discussion, and any contribution may be of value.

The authors of this paper have, therefore, made use of the occasion offered by the natural conditions of our country—with its more or less isolated valleys—for making an investigation on the occurrence and heredity of twin-births. The population of such a valley may often genetically be considered as forming one great family; by making use of church-registers and archives it has been possible, therefore, to collect a material genealogically accurate and at the same time large enough to form the basis of statistical treatment.

If after all, our results will not be found to solve definitely the question, we believe this to be due to the nature of the problems before us more than to the material or to the methods used.

(b) *Material.*

During earlier investigations on Norwegian peasant families conducted by the "Institut for Arvelighetsforskning" of the University of Oslo, we have been struck by the fact stated also in other countries (Prinzing 1907, Puech 1874), that in the families of one parish twin-births may occur considerably more frequently than in those of other valleys, although this difference does not necessarily make its appearance in the statistical picture of the parish taken as a whole. Thus, in one great family from Sogn, in which all births had been noted for 73 marriages, two marriages only (2.74 per cent.) were found with twin-births. Another family from Drangedal with 310 marriages investigated had no twin-birth at all. A third large family, or rather a series of genetically intertangled families from Ringebru, with 380 fully investigated marriages, proved in comparison with the other two to represent a *twin-family*, showing twins (or triplets) in no less than 71 (19.5 per cent.) of all marriages.

This family from Ringebru was taken as a starting-point of our investigations upon the heredity of a predisposition for twin-production, and a preliminary account (Bonnevie 1919) has been given of the main results so far reached. Later on the work has been continued through an investigation also of a series of families living within Meldal and Rennebru, partly also in Orkedalen, these three parishes forming together the valley of the river Orkla, which falls into the Trondhjemsfjord.

The frequent occurrence within these families of twin- (or triplet-) births had been pointed out by Dr Stören (1901), the district-physician of Meldal, who in various ways has rendered good services to the knowledge of the population of his district.

As in Ringebru, our investigations in Meldalen and Rennebru have been made partly in the field, during the summers of 1919 and 1920, much information having been obtained through public officers and from representatives of the families investigated. Every information, thus obtained has, however, been controlled through the church-registers, and by far the most important part of our pedigrees are based upon the church-registers only, or upon the "Records of division of inherited property," both going back to the second half of the seventeenth century. In our pedigrees, therefore, the descent of twin-pairs has been traced back to the time mentioned, while side-branches of the same families were also worked out. Referring to the oldest ancestors, the families have been grouped under separate headings (in Meldal fam. 1-21, in

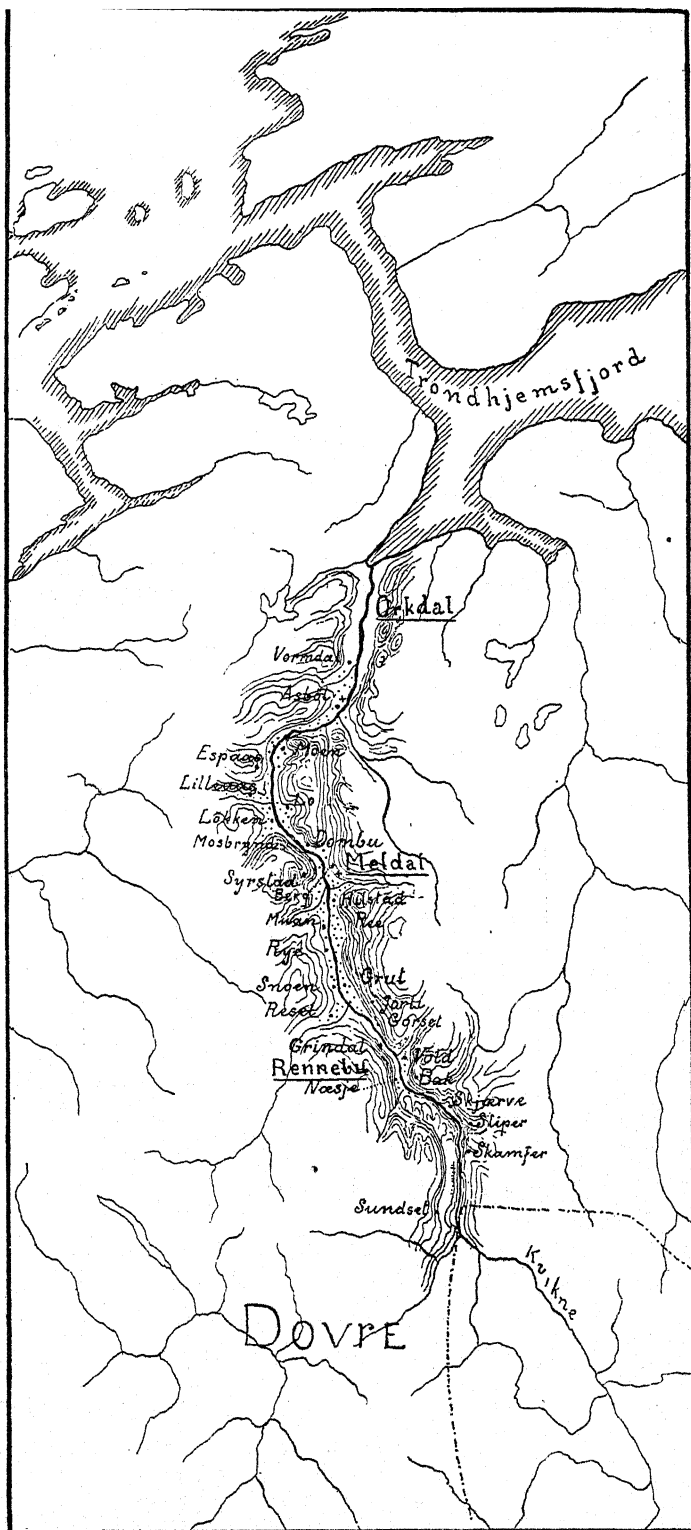


Fig. 1. Map showing the long and narrow valley including the parishes of Rennebu, Meldal and Orkdal, with names of farms where important twin-families have been,

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Rennebu fam. 1-14). But it is indeed very difficult to tell how much belongs to one family and how much to another. One gets the impression that the native population of the whole valley might, if the church-registers had reached further back, have been acknowledged as descending from a relatively very small number of families, once immigrated into the valley. The pedigrees published in this paper all represent more or less restricted fragments of the full family records, which are preserved in the "Institut for Arveligthesforskning," Kristiania.

It would, of course, be of interest to compare our results also with those of other investigators and from other countries. And, indeed, in the very extensive pedigrees published by Lundborg (1913) on a Swedish family from Blekinge we have before us a material which, with regard to twin-births, may on several points be used for a comparison, and which may statistically be added to the results from our Norwegian family-material.

The family-material used in the following discussion consists,

Material investigated.

	Populations	Marriages	Births	Multiple births:		
				On the pedigrees	Not on pedigrees	Sum total
Family-Material	Meldalen	904	3544	101	52	153
	Rennebu	677	2733	107	52	159
	Ringeby	905	3877	122	0	122
	[Blekinge ^x)		1880	29	3	32]
	Sum total		12034	359	107	466
Statistics	Norway 1916-17		125950			1848
	Women's Hosp. 1876-1916					537

Sum total: 2851 Multiple births

^x) Extracted from Lundborg (1913)

TABLE I. Survey of material investigated.

therefore, of elaborate pedigrees from three Norwegian parishes: Meldal, Rennebu, Ringeby, use having been made also of the Swedish Blekinge family, described by Lundborg. Besides these large pedigrees we have also a series of small families from the same parishes, which probably but not with absolute certainty are related to the others. As will be seen from Table I the whole family-material comprises thus 12,034 births with a sum total of 466 twin-births, still-born twins here included.

Besides the families from the districts mentioned, an isolated case of multiple twin-production from Elverum, one woman having no less than eight twin-births, will also be considered.

Several questions, as for instance the relations between mono- and dizygotic twin-births, need for their solution a comparison with the statistics of other populations. We have obtained such material from two sources, making use, first by the kind permission of the Director of the "Women's Hospital," Kristiania, Professor Kr. Brandt, to investigate the registers of this hospital for a century with 537 twin-births. We obtained also leave from the "Central Office of Statistics" for a special research within the Norwegian population with regard to twin-births for each of the two years 1916 and 1917, including 1848 twin-births, living or dead. We are greatly indebted to the Directors of these two institutions for their assistance.

(c) *Indications of heredity.*

Indications of heredity, as playing a part in the production of twins, occur so often in scientific literature as well as in general experience, that no special argument on that score is needed.

A few figures may suffice.

As will be seen from Table V, line 9, col. 1 (p. 59), multiple births occurred within the Norwegian population in the two years 1916-17 in a percentage of 1.46, which may be considered a normal rate of twin-births in Norway. The next column (2) of the same line proves the percentage of twin-births within Meldal and Rennebu for the period 1750-1895 to be even lower than the normal percentage of Norway, viz. 1.24 per cent. But, after all, the rate of twin-births, within each of the great Norwegian families investigated, from Meldal, Rennebu and Ringeby (Col. 3-5), is raised to a percentage of 2.85-3.91. The whole Norwegian family-material taken together (col. 6) shows, therefore, a percentage of twin-births of 3.25, more than twice that of the Norwegian population.

In all the families investigated a thorough analysis has proved the great majority of twin-births to be accumulated upon certain genetical lines, while other great branches of the families may show no single multiple birth. A few such twin-lines extracted from our full pedigrees as well as one extracted from the pedigrees of Lundborg are shown in Pls. XV-XVII. Calculation from such lines (col. 7) shows a further rise of the twin-birth rate up to 8.23 per cent.

Such a gradual rise of the percentage of twin-births, when passing from the whole population to special families, and within these to more

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isolated lines, proves without doubt the hereditary nature of multiple births.

That tendency to twin-births does not exist within all families is on the other hand demonstrated by the fact, already mentioned, that in two other great families of Norwegian peasants, from Sogn and from Drangedal with 313 and 800 births respectively, twin-births occurred in the first family in two marriages only, or in 0.6 per cent. of all births, while in the other no single twin-birth has been noted.

The figures here quoted support the view maintained by earlier authors that *the existence in man of a hereditary predisposition for twin-births*¹ is obvious. Difficulties are however, as already mentioned, raised from the very nature of the twin-births, these representing two groups according to whether the twins are *monozygotic*, or *dizygotic*. The nature of these two groups of twin-births is entirely different, while the two kinds of twins themselves are not always easily distinguishable². The first, and perhaps the greatest, difficulty to be overcome in an investigation upon the heredity of twin-births is therefore, that of determining the true nature of the twin-material before us.

The importance of such distinction is evident, a conclusion drawn with regard to the heredity of one kind of twin-births having no value whatever for our understanding of the occurrence of the other. One of them may *a priori* be supposed to be hereditary and the other perhaps not, or they may both prove to be caused by some hereditary tendency; but if so, their type of heredity may again be entirely different. Many of the contradictions found in literature with regard to the heredity of twin-births will certainly prove to be due to this confusion.

CHAPTER II.

NATURE OF THE HEREDITARY PREDISPOSITION OF TWIN-FAMILIES.

Before entering upon a study of the type of heredity we must decide, if possible, the nature of the twin-births characteristic of and hereditary in the family-material before us. We require a *distinction between mono- and dizygotic twin-births*; and it will further be of interest to investigate the theory of Puech (1874) as to a *possible causal connection between general fertility and frequency of twin-births*.

¹ Statistical investigations of a similar predisposition in sheep have been undertaken with positive results by Rietz and Roberts (1915) and Wriedt (1917)—and less conclusively for horses, by Robertson (1913) and by Stroeve (1917).

² Taking his starting-point in the phenotypical similarity Fisher (1919) has made a vain attempt to eliminate the genetical difference between mono- and dizygotic twins.

(a) *Mono- or dizygotic twin-births.*

No clinical observations are at our disposal with regard to the true nature of the twin-births of the peasant families before us. The question whether they should be considered as mono- or dizygotic must, therefore, be solved through a statistical study only, based on the fact that in twin-births the sexual-proportion has repeatedly been stated to be somewhat the same as in single births.

Upon this basis Weinberg (1902) has introduced his "Difference-method," according to which the number of mono- and dizygotic twin-births within a given population can be approximately decided.

This "difference-method" is founded upon the fact that *monozygotic* twin-pairs are always of the same sex ($\delta\delta$ or ♀♀), while among *dizygotic* twins the sexual-proportion is supposed to be the same as among sisters and brothers in general, that is approximately an equal number of males and females, the distribution of whom on the twin-pairs should expectedly be that of $1 \delta\delta + 2 \delta\text{♀} + 1 \text{♀♀}$.

If therefore in a given population of twin-pairs to the number of bisexual twin-pairs is added a similar number of the unisexual ones, we should get the whole number of dizygotic twins, while the remainder of unisexual twin-pairs would represent the *monozygotic* ones.

The same method has, independently been used also by Cobb (1915) while Duncker (1915), without knowledge of Weinberg's method, has introduced a more elaborate calculation in which allowance is made also for the surplus of males expressed in the general sexual-proportion of man ($106 \delta : 100 \text{♀}$).

Such statistical methods can of course, as acknowledged also by the authors, give fully reliable results only when working with a large material; the smaller the figures, the less exact the results. In order to test the value of both methods we have, from the literature, collected a number of cases in which information has been given in one and the same material of the distribution of sex as well as of the clinical results with regard to the secundines (see Table II, p. 138).

Full informations have been given by Rumpe (1891) for 166 twin-pairs as well as by Meyer (1916) for 250 pairs. To these are in Table II added also a number of 71 twin-pairs, for which full informations have been given by Women's Hospitals in Kristiania. Other authors, as Spaeth (1860), Brém (1891) Quenzel (1898), Schapiro (1912), Neuhäuser (1913) have given similar information without, however, specifying the numbers of male and female twin-pairs, within the group of unisexual ones. This

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difference is of no significance for our testing of the "difference-method," while for making use of the calculation of Duncker the exact number of

Secundines investigated										Sex of Twin-pairs				Nature of Twin-births			
Chorion										Mono		Di-		Mono-xygical		Di-xygical	
Single					Double					Sum	Total	Sum	Total	Observed	Diff. meth.	Obs.	Diff. meth.
♂♂	♀♀	Sum	♂♂	♀♀	Sum	♂♂	♀♀	Sum	Total	Mono	Di-	Sum	Total	abs.	%	abs.	%
36	29	65	31	16	54	101	166	112	54	166	54	112	54	65	34.2	58	34.9
22	17	39	36	32	123	211	250	127	123	250	123	127	123	39	15.6	4	1.6
15	5	20	18	15	18	51	71	53	18	71	18	53	18	20	28.2	35	49.3
73	51	124	105	63	195	363	487	292	195	487	195	292	195	124	25.5	97	20.0 ^x
		31	38	36	94	125	89	36	125	89	36	125	89	31	24.8	53	42.4
		25			85	110	79	47	126	79	47	126	79	25	22.7	32	25.4
		37	64	78	142	179	101	78	179	101	78	179	101	37	20.7	23	12.8
		36			123	159	104	73	179	104	73	179	104	36	22.6	29	16.2
		6			109	115	71	51	132	71	51	132	71	6	5.2	20	16.4
		259			916	1175	736	482	1218	736	482	1218	736	259	23.0	254	20.9
										</							

The percentage will therefore, more than the absolute numbers of mono- and dizygotic twin-births, serve as a basis of comparison between the results of observation and of calculation.

As will be seen from Table II, the percentage of dizygotic twin-births found through the "difference-method" of Weinberg may in the various small populations vary very considerably from that really observed (by Spaeth 57.6 per cent. dizygotic twin-births instead of 75.2 per cent.; Meyer 98.4 per cent. instead of 84.4 per cent., etc.). But in the large material obtained through adding all the figures given, the results of the "difference-method" appear to be very satisfactory, the calculated numbers of di- and of monozygotic twin-pairs differing little more than 1 per cent. from those observed (79.1 per cent.: 78.0 per cent. and 20.9 per cent.: 22 per cent.).

The material represented in the three first lines of Table II, including special references to the numbers of male and female twin-pairs, has been calculated also according to the method of Duncker¹. It appears, however, that in this material of 487 twin-pairs the difference-method of Weinberg brings us nearer to the actually observed figures than does the method of Duncker. Even if, theoretically, a more exact result should be expected from a method of calculation, which takes into consideration also the sexual-proportion of the population investigated, experience has proved that because of the relatively great mortality of twin-embryos, the sexual-proportion of twin-populations may be found to vary within a range considerably wider than that of single births. The possible advantage of the method of Duncker will, therefore, for its documentation need a very large material.

According to the above considerations the "difference-method" of Weinberg has been used for all calculations with regard to mono- and dizygotic twin-births. The results given in Table II also show the obvious fact that the calculated values can only be approximate and, of course, the less exact, the smaller the population considered. The objection made by Meyer (1916, p. 328), on account of 250 twin-pairs in which the sex as well as the number of chorions has been stated (cf. Table II), against the validity of the difference-method will therefore scarcely prove justified.

As first proved by Duncan (1865), and as demonstrated also in our preliminary report (Bonnievie 1919, Figs. 2-3) upon the Ringeby family

¹ The calculation of Duncker is based upon the formula $n' = \frac{2f''}{1-d^2}$, in which n' represents the number of dizygotic twin-births, f'' the number of bisexual twin-pairs ($\sigma\varphi$), while d is the sexual-proportion of the twin-population investigated (*loc. cit.* pp. 507-511).

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there seems to be some correlation between the age of the twin-mother and the number of twin-births. Our Ringebu investigation has also supported the view maintained by Rumpe (1891) that upon this point a difference exists between mono- and dizygotic twin-births.

Here again, therefore, our whole material of twin-births has for analysis been grouped according to the age of the twin-mother, as will be seen in Table III, where the twin-births are arranged in groups corresponding each to five years of age of the mother, while in Table III *a-b* the full analysis (per year) of the same material will be found. All curves, of Figs. 2-6, have been constructed upon series found in Table III.

Ser.	Age of mother:	-19	-24	-29	-34	-39	-44	-49	n	A	p.e
Norway, 1916-1917	1	<u>Births</u>	2697	23228	32924	30172	22046	11744	1761	124572	31,03 ± 0,004
	2	<u>Twin - births</u>	8	187	406	472	438	205	20	1736	32,28 ± 0,145
	3	in % of 1	0,29	0,8	1,24	1,56	1,99	1,74	1,13		
	4	Mono - zygotical	6	75	128	126	76	55	12	478	31,45 ± 0,243
	5	in % of 2	75	40,1	31,5	29	17,4	26,8	60	27,53%	
	6	Di - zygotical	2	112	278	346	362	150	8	1258	32,6 ± 0,164
	7	in % of 2	25	59,9	68,5	71	82,6	73,2	40	72,47%	
Women's Hosp. 1816-1916	8	<u>Twin - births</u>	7	117	175	112	95	29	2	537	29,38 ± 0,26
	9	calculated for n=1736	22,6	378,2	565,7	362,1	307,1	93,8	6,5	1736	
	10	in % of 1	0,84	1,63	1,72	1,2	1,4	0,8	0,37		
	11	Mono - zygotical	7	45	75	27	19	5	2	173	26,63 ± 0,428
	12	in % of 8	100	38,5	42,9	17,9	20	17,3	100	32,22%	
	13	Di - zygotical	0	72	100	92	76	24	0	364	30,69 ± 0,309
	14	in % of 8	0	61,5	57,1	82,1	80	82,7	0	67,78%	
Family - material	15	<u>Twin - births</u>	5	43	73	89	108	57	5	380	32,84 ± 0,324
	16	calculated for n=1736	22,84	196,4	333,5	406,6	493,5	260,4	22,8	1736	
	17	in % of 1	0,83	0,846	1,013	1,35	2,24	2,21	1,3		
	18	Mono - zygotical	1	4	19(+1)	14	7	+5	1	41(+1)	28,15 ± 0,766
	19	in % of 15	20	9,3	27,4	15,7	6,5	0	20	11,05%	
	20	Di - zygotical	4	38(+1)	50(+3)	74(+1)	96(+5)	60(+2)	4	326(+12)	33,55 ± 0,344
	21	in % of 15	80	90,7	72,6	84,3	93,5	100	80	88,95%	
	22	Sum total of twin births	20	347	654	673	641	291	27	2653	
Norway	23	Births (n=1000)	21,6	186,9	264,2	242,2	176,9	94,2	14,1	1000	
	24	Twins (n=1000)	4,6	107,7	233,9	271,9	252,3	118,1	11,5	1000	

TABLE III. Frequency of births, twin-births, mono- and dizygotic twin-births, of the various groups of material investigated, arranged according to the age of the twin-mothers. The material upon which the series of this table (except ser. 1 and 23 taken directly from the "Official Statistics") have been based is found in Table III *a-b*, printed at the end of this paper. (pp. 187-188.)

The figures added in () in ser. 18 and 20 represent a small number of twin-births whose sexes are unknown, but which are included in the figures of ser. 15; they have been distributed between the groups of mono- and dizygotic twin-births according to the relative size of the latter.

As already mentioned above, our material includes, besides the family-material, the twin-births occurring within a period of a hundred years in the Women's Hospital of Kristiania, as well as of those of the

Twin-material (cf. Table III).

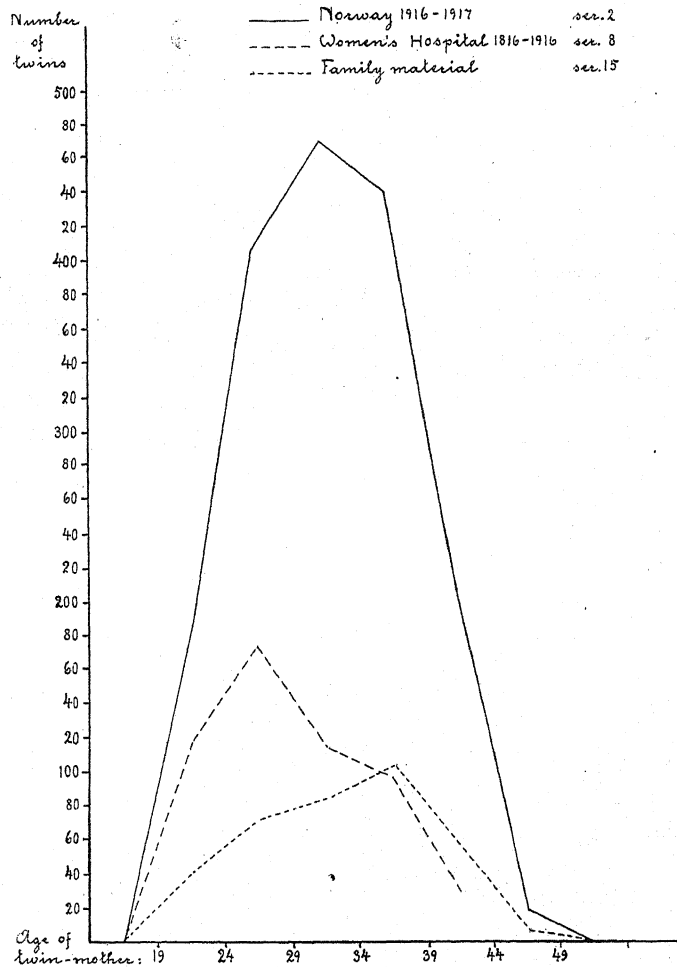


Fig. 2. Curves of twin-births, arranged according to the age of the mothers, within each of the three groups of material investigated.

whole Norwegian population in the two years 1916-17. Each of these three groups of our material is represented in one of the curves of Fig. 2.

These curves show very characteristic differences. The maximum of twin-births in the clinical material falls between 24 and 29 years of age

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of the twin-mother; in that of the Norwegian population the greatest number of twin-births is found with mothers 29–34 years old, while in the family-material the maximum number of twins are borne by mothers 34–39 years old.

The curve of the whole population being based upon no less than 1736 twin-pairs¹ and from a wholly representative material should here be taken as the norm, in relation to which the characteristics of the two other curves should be considered.

The peculiarities of the clinical material are easily understood when remembering, what has been repeatedly mentioned also by previous authors, that such material will always be of a more or less select nature. In the clientèle of a Lying-in Hospital young women will as primiparae always be more numerous proportionally than in the population as a whole—and the absolute number of twin-births will in clinical material be raised also for the reason that dangerous complications occur more often in multiple than in single births.

Of more general interest are the peculiarities of the family-material. But before entering into an analysis of this question, it will be necessary to study more thoroughly the occurrence and the nature of the twin-births in the whole Norwegian population, considering both the relations of twin-births to births in general, and the statistical relations between mono- and dizygotic twin-births.

The relation between *births* and *twin-births* in Norway is plainly demonstrated in Fig. 3, the two curves of which are based upon the series 23 and 24 of Table III, or in other words upon the two series for births (ser. 1) and for twin-births (ser. 2) both reduced to the same size, viz. $n = 1000$.

Both series are seen to form approximately symmetrical curves falling steeply down towards the beginning as well as towards the end of the fertile age of the mothers. But at the same time the curve of the twin-birth is seen to be independent of the birth-curve in so far as its maximum is reached at a later age of the mothers, between 29 and 34 years instead of the 24–29 years maximum of the birth-rate. In Table III the average age of the mothers is shown to be for the birth-curve (ser. 1) 31.03 ± 0.004 , while for the twin-birth curve (ser. 2) it is 32.28 ± 0.145 .

Such independence between the two curves proves, in full agreement with the view maintained by Duncan (1865), the existence in the population of a *predisposition for twin-births* varying with the age of

¹ Of the total 1878 twin-pairs (cf. Table I), 112 pairs for which the mother's age was unknown, have been excluded.

the mothers, while in its results dependent also upon the birth-rate characteristic of each age-group.

In order to get a clear picture of these relations it will be necessary to consider not only the absolute numbers of multiple births which must necessarily be strongly influenced by the birth-rate, but above all the relation between births and twin-births within each age-group of the

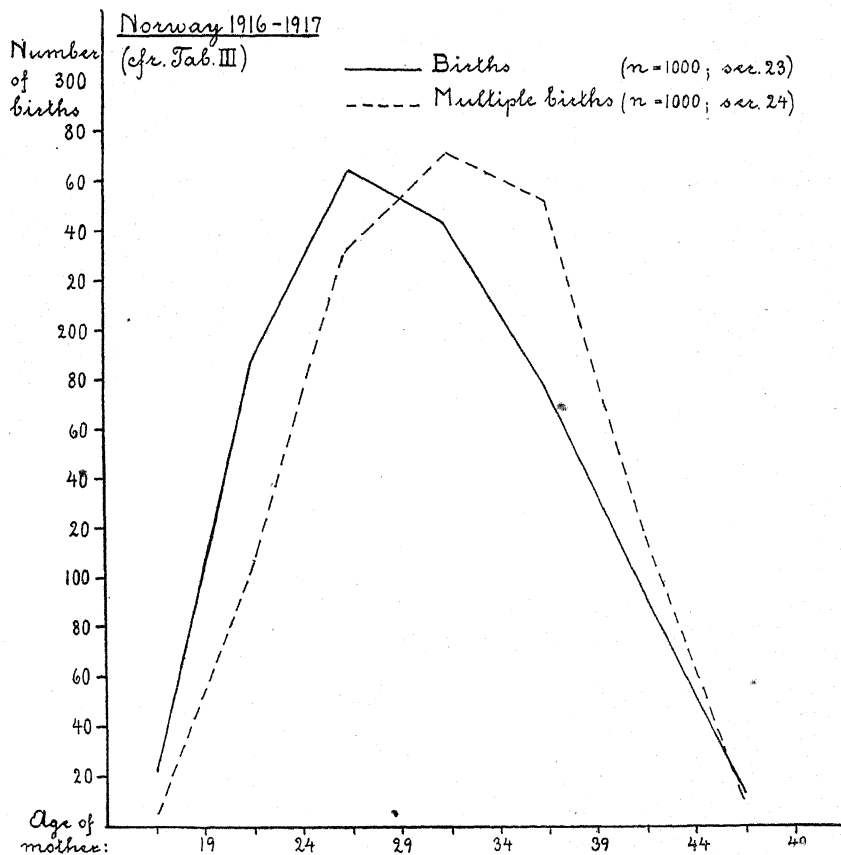


Fig. 3. Curves of births and twin-births in relation to the age of the mothers, within the population of Norway 1916-17. For facilitating the comparison the frequencies of both series have been reduced to $n=1000$.

mothers, that is the number of twin-births expressed in percentage of the total number of births. A curve of this kind is given in Fig. 4 *b*, based on series 3 of Table III, while in Fig. 4 *a* the absolute numbers of twin-births are demonstrated. The predisposition for twin-births is here seen to be very slight among young mothers but to increase quite regularly with

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their growing age, the curve reaching its maximum in mothers, 34 and 39 years old; after this time the disposition for twin-births seems to be slowly diminishing.

Norway 1916-17 (cf. Table III a, ser. 2, 4, 6).

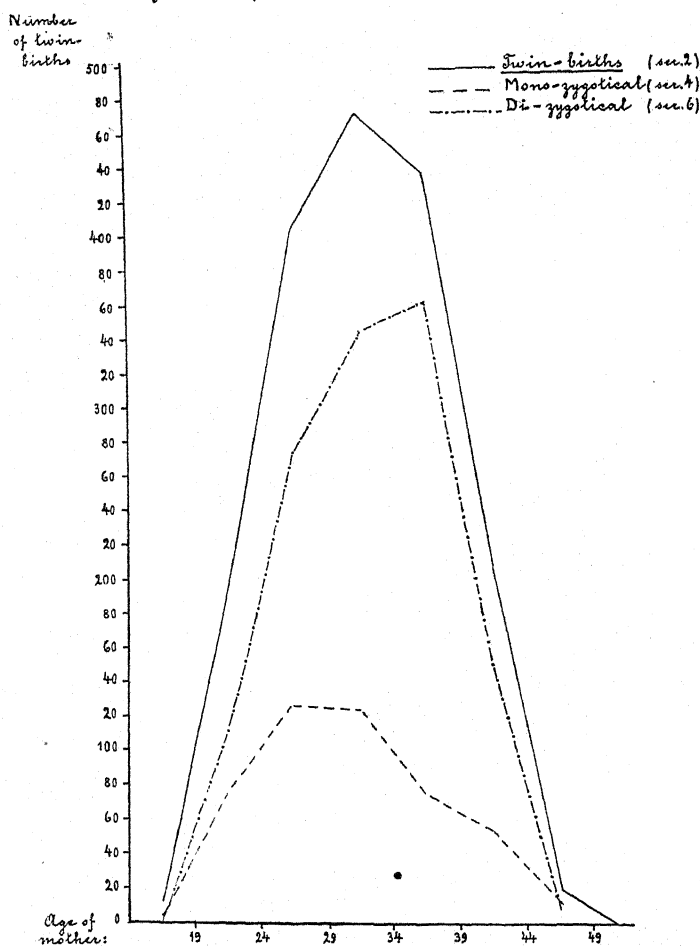


Fig. 4 a. Analysis of the occurrence of twin-births (including still-born) in the Norwegian population: the frequencies of monozygotic and of dizygotic twin-births as well as of their sum total within the various age-periods of the mothers.

In the two lower curves of Fig. 4 a-b are demonstrated the results of an analysis of the twin-births, according to the "difference-method" of Weinberg, the material having been divided into two groups representing the *mono-* and *dizygotic* twin-births respectively.

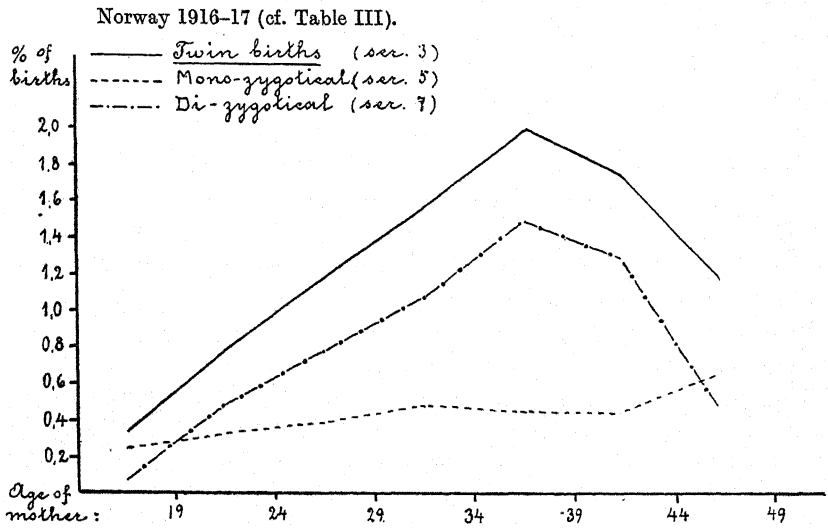


Fig. 4b. The same frequencies as in Fig. 4a expressed in per cent. of the number of births within each age-period.

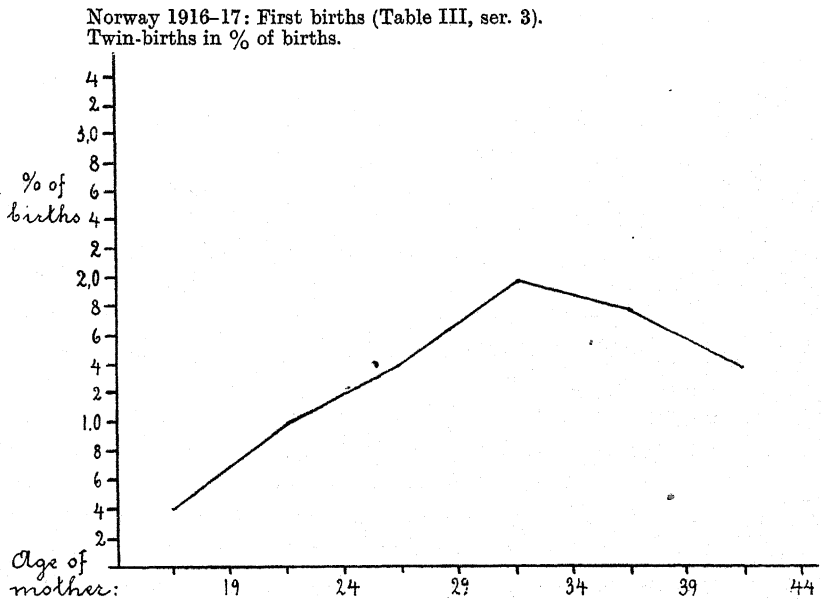


Fig. 4c. Frequency of "first births" arranged in the same way as in Fig. 4b.

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The absolute numbers of each group shown in Fig. 4a are found in series 4 and 6 of Table III. A characteristic difference is seen to exist between them, the curve of monozygotic twin-births reaching its maximum already in mothers 24–29 years old, with an average of 31.45 ± 0.243 , while that of dizygotic births is constantly rising up to an age of 34–39 (av. 32.6 ± 0.164). The sum totals of mono- and dizygotic twin-births make 27.53 per cent. and 72.47 per cent. of the whole number of twin-pairs.

A very interesting supplement to this picture is given in the two lower curves of Fig. 4b proving the *regular increase and fall* of the disposition for twin-births to be due to the *dizygotic* twin-births only, this curve (Table III, ser. 7) following very closely the course of the main curve. The tendency for *monozygotic* twin-births (Table III, ser. 5) seems, on the other hand, to be *independent of the age of the mother*, the curve here taking an approximately horizontal course. Irregularities at the extreme ends of these curves will be of no real importance, considering the rapid decrease in the absolute number not only of multiple births but of births in general by mothers more than 40 years old. This result supports the conclusion drawn by Rumpe (1891) from his material of some 160 clinically investigated twin-births (s. 346) “dass zweieiige Zwillinge vorwiegend von Müttern im vorgeschrittenen Alter (über 25 Jahren) geboren werden,” and further that “eineiige Zwillinge...in jedem Geschlechtsalter gleich oft beobachtet (werden).”

The dependence upon the age of the mother here demonstrated for the predisposition of twin-births might of course mean either a *direct* causality existing between age and twin-births, or an *indirect* one if not the age itself but the number of previous births by the same mother should prove to be decisive for the development of such predisposition. This question has, as already mentioned, been raised by Duncan (1865a–b) who, after some hesitation in his first paper, finishes by declaring in the second (p. 929) that: “the increased frequency of twin-bearing as the number of the pregnancy increases may...be now regarded as a law of the production of twins.”

A definite solution of this question will, of course, meet with the difficulty that a high frequency will never be met with except in older women; the parts played by each one of these causes will, therefore, not easily be decided. The only way of eliminating one of the two factors will be that of considering the primiparae as an isolated group, women of all ages (within the period of fertility) here being met with.

Table IV gives our material for investigating the twin-births among

1st pregnancies in relation to the age of the mothers, illustrated for the whole population of Norway also in the curve of Fig. 4 c.

In this curve (cf. series 3 of Table IV) the percentage of twin-births among 1st pregnancies is seen to augment with the age of the mothers, following very near the same line as the curve of the general predis-

<u>First births</u> considered alone		Series	Age of mother. [years.]						n
			-19	-24	-29	-34	-39	-44	
Norway 1916 17	Births	1	1457	10782	8676	3591	1286	432	26271
	Mult. births	2	6	107	121	71	23	6	334
	" in % of 1	3	0,41	0,99	1,39	1,98	1,79	1,39	
Family mate- rial	Mult. births	4	2	18	17	11	6	5	59
	" in % of 1	5	0,15	0,17	0,19	0,3	0,47	1,16	
	Dizygoteal	6	2	12	16	12	6	6	54
	Monozygoteal	7	0	6	1	-1	0	-1	5

TABLE IV. Frequency of "first births" in the Norwegian population, and in the family-material, arranged according to the age of the mothers.

position for twin-births (Fig. 4 b). The slight difference between both curves with regard to mothers 34-39 years old will not surprise when taking into consideration the low number of primiparae among mothers of this age.

It seems, therefore, absolutely certain that the age of the mothers plays an important and direct part in the development of a predisposition for twin-births. This fact does not, it is true, exclude the possibility that a high number of pregnancies may also be of some significance. The similarity of the two curves of Fig. 4 b and c, proves, however, that not much room is left for any influence other than that of the age of the mother.

The results here reached with regard to the predisposition for twin-births existing in the Norwegian population, and demonstrated in the curves of Fig. 4 a-c, may serve as a basis of comparison when turning, now, to a consideration of our *family-material*.

Fig. 5 a, the curves of which are based upon series 15, 18 and 20 of Table III, gives an illustration of the absolute numbers of twin-births in the family-material as well as the numbers of dizygotic and monozygotic twin-births, calculated according to the "difference-method." The most characteristic features of these curves will be seen *first* in the relatively very high age (34-39 years) of the mothers giving the maximal frequency of twin-births. *Secondly* the curve of dizygotic twins is

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extraordinary with regard to its height, as well as with regard to its shape. It covers in the family-material no less than 88.95 per cent. of the whole field, while 72.47 was the percentage of dizygotic twin-births in the whole population; and the shape of the dizygotic curve of the family-material is, especially for the older women, very near the same as that of all twin-births taken together, so near, indeed, that we are justified in saying that the dizygotic twin-births are here alone responsible for the deviation from the typical curve (Fig. 4a) of the whole population.

Family-material (cf. Table III).

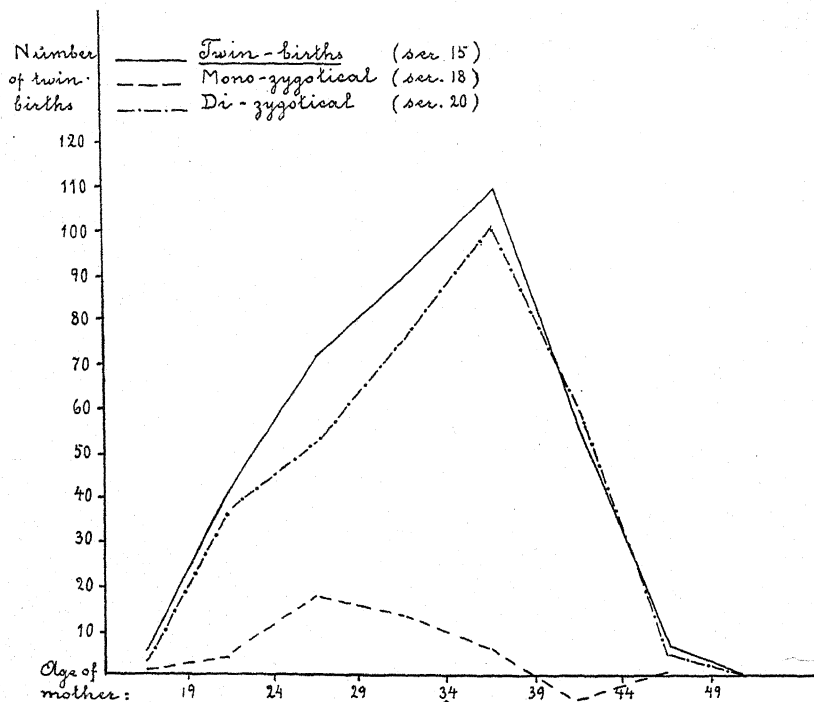


Fig. 5a. Twin-births (still-born included) in the "family-material" arranged as in Fig. 4a.

These characteristic features of the family-material are even more clearly demonstrated in the curves of Fig. 5b, showing the number of twin-births within each quinquennial age-period of the mothers (Table III, ser. 15) expressed in percentage of the "normal birth-rate" of the Norwegian population, as found in series 1 of Table III. In order to facilitate a comparison with our results from the whole population, the series of twin-births in the family-material has before finding the per-

centages been calculated for $n = 1736$, that is for a total number of twin-births like that of the Norwegian population (Table III, ser. 16).

The predisposition curves of the family-material, thus shown in Fig. 5 *b*, emphasize once more, and with all desirable clearness, the heavy preponderance of dizygotic twin-births. We find here a predisposition for twin-births, which already in young mothers largely surpasses that of the whole population and which rises rapidly, from an age of about 24 years, to a very conspicuous maximum reached, as in the curve of Fig. 4 *b*, at the age of 34–39 but lasting in the family-material also during

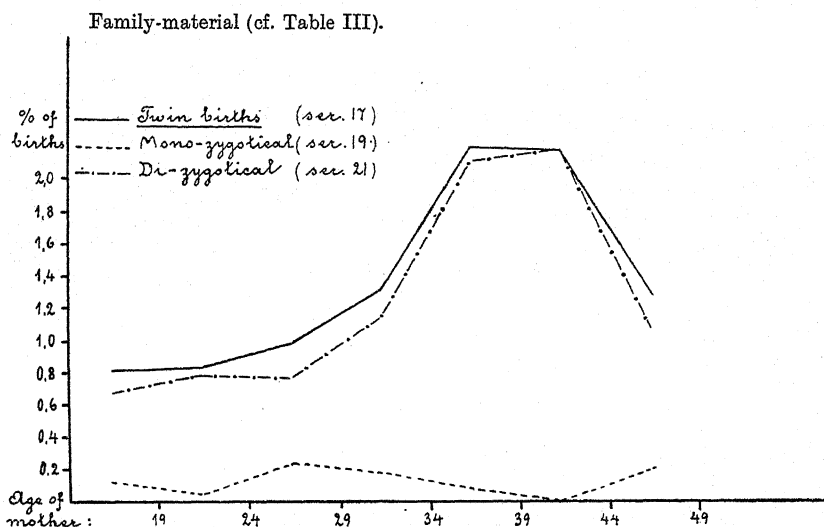


Fig. 5 *b*. Twin-births in the "family-material" expressed in per cent. of the "normal birth-rate" (see text).

the following 5-years period. The rapid fall of the curve between 44 and 49 years should, as mentioned above, not be considered as reliable. The very characteristic course of the main curve is here again closely followed or, more correctly spoken, it is evidently determined by the curve of dizygotic twin-births, while that of the monozygotic births runs approximately horizontally, that is, it shows the same independence with regard to the age of the twin-mothers as was the case in the whole population.

Fig. 5 *c*, based upon series 5 of Table IV, demonstrates finally, for the twin-births among 1st pregnancies the same characteristic feature: a very conspicuous rise of the predisposition for twin-births with growing age of the mothers. A comparison between series 4 and series 6 of Table IV

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proves, further, that even among the 1st pregnancies the dizygotic twin-births seem to be decisive for the shape of the curve.

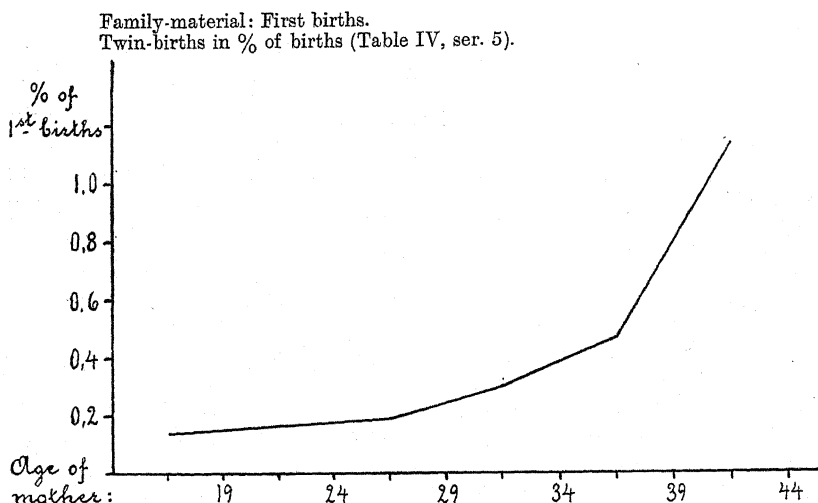


Fig. 5c. Twin-births among "first-births" in the "family-material," expressed in per cent. of births.

We feel, after the above demonstration, fully justified in considering an *increased predisposition for dizygotic twin-births* to be the character inheritable in our twin-families. Such predisposition must, however, as generally maintained also by earlier investigators, be due to some characteristic feature in the ovary of the *twin-mother* causing the existence, simultaneously, in her uterus of two (or more) fertilised eggs, while, so far as dizygotic twin-births are concerned, the father of the twins will be of no significance.

The question about monozygotic twin-births, their nature and their possible heredity, will in what follows be left out of consideration, our whole attention being turned towards the *twin-mothers as representatives of an hereditary predisposition for dizygotic births*.

(b) *Dizygotic twin-births in relation to fertility.*

As already mentioned above, Puech (1874) strongly emphasized his view of a causal connection existing between multiple births and the general fertility of an individual woman, of a race or of a certain district.

A priori, such causal connection would for dizygotic twin-births, seem very probable. A predisposition to double ovulations which has its structural basis in the existence in the adult ovary of an unusual number

		1	2	3	4	5	6	7	
	Line	Norway 1916-1917	Meldal and Rennebu 1750-1895	Meldal Fam: 1, 21	Rennebu Fam: 1-12	Ringebu Fam: 1-22	Family Material Sum Total	Twin- lines	
Individuals born.	Sum total	1	127844	13155	3645	2840	4000	10485	1618
	Sex known	2	127844	6589	3560	2832	3801	10193	1605
	♂	3	65745	3311	1781	1432	1936	5169	761
	in % of 2	4	51,42	50,25	50,03	50,56	51,46	50,71	47,41
	♀	5	62099	3278	1779	1400	1845	5024	844
	in % of 2	6	48,57	49,75	49,97	49,44	48,54	49,29	52,59
Births		7	125978	12994	3544	2733	3878	10155	1495
Multiple births.	Sum total	8	1848 ^(*)	161	101	107	122	330	123
	in % of 7	9	1,46	1,24	2,85	3,91	3,15	3,25	8,23
	Sex unknown	10	112	3	13	2	6	21	7
	Sex known	11	1736	158	88	105	116	309	116
	♂ ♂	12	606	49	23	20	44	87	32
	♀ ♀	13	501	53	32	32	26	90	32
	Monosexual	14	1107	102	55	52	70	177	64
	in % of 11	15	63,67	64,56	62,5	49,52	60,35	57,28	53,17
	Di-sexual	16	629	56	33	53	46	132	52
	in % of 11	17	36,33	35,44	37,5	50,48	39,65	42,72	44,83
	Mono-zyg.	18	478 ⁽³²⁾	46 ⁽¹⁾	22 ⁽²⁾	+ 1	24 ^(1,2)	45 ⁽³⁾	12 ⁽⁴⁾
	in % of 11	19	27,53	29,11	25,0	+ 0,95	20,69	14,56	10,35
	in % of 7	20	0,40	0,36	0,71	+ 0,04	0,65	0,47	0,87
	Di-zyg.	21	1258 ⁽⁸²⁾	112 ⁽⁷⁾	66 ⁽¹⁰⁾	106 ⁽²⁾	92 ^(4*)	264 ⁽¹¹⁾	104 ⁽⁶⁾
	in % of 11	22	72,47	70,89	75,0	100,95	79,31	85,44	89,65
	in % of 7	23	1,06	0,88	2,14	3,95	2,50	2,78	7,36

^{*)}18 of which are triplets.

TABLE V. Statistical data regarding births (lines 1-7) and twin-births (lines 8-23) in the whole Norwegian population (col. 1), in the whole valley of Meldal and Rennebu (col. 2), in each of the twin-families of Meldal, Rennebu and Ringebu (cols. 3-5), in the whole family-material (col. 6) and in special twin-lines (col. 7).

The following facts should be noted:

The numbers of births (line 7) have been found by subtraction of the sum total of multiple births (line 8) from that of individuals born (line 1), remembering that each twin-birth makes the number of individuals surpass by 1 (or in case of triplets by 2) the number of births.

The numbers of mono- (line 18) and dizygotic (line 21) twin-births have been calculated through the difference-method of Weinberg on the numbers of mono- and bisexual ones (lines 14 and 16).

The twin-births, whose sex was unknown (line 10) have been added in () to the groups of mono- and dizygotic twin-births (line 18 and 21), distributed between these groups according to their relative size. These figures have been included in the numbers of mono- and dizygotic twin-births when calculating their percentage of the total number of births (lines 20 and 23).

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	Sex	Number of Births																n	A ± se	Difference
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16			
Twin-families in % of n.	Malsal	4	19	37	52	76	93	50	55	23	20	9	3	1				442		
	Rennebu	2	11	67	90	63	53	37	22	8			2	1				356		
	Ringebu	7	14	29	54	44	57	51	46	29	25	12	5	3	3	1		380	A ₁	
	Sum	11	35	77	173	210	213	154	138	74	53	21	10	5	3	1		1178	6.09 ± 0.065	
Twin-families in % of n.	Malsal, Fam. 17	0,9	3,0	6,5	14,7	17,8	18,1	13,1	11,7	6,3	4,5	1,8	0,9	0,4	0,3			100		A ₂ -A ₁ = 0.69 ± 0.23
	Ringebu A-D				4	8	3	3	6	2	3	1						30		
	Sum	2	9	13	9	9	16	15	9	8	3	2	1					96	A ₂	
	in % of n.	2	9	17	12	12	19	21	9	10	6	2	2					126	6.78 ± 0.222	
Twin-families in % of n.	Malsal	1,6	7,1	13,5	13,5	9,5	15,1	16,7	7,2	7,9	4,7	1,6	1,6					100		
	Rennebu			2	9	9	8	9	4	1	2	1						45		A ₃ -A ₂ = 0.67 ± 0.19
	Ringebu			1	5	12	13	7	2	5	1	1						47		
	Sum			1	2	6	6	3	4	6	3	1	1					33	A ₃	
Twin-families in % of n.	Malsal	2	9	27	28	18	15	15	5	4	1	1						125	6.76 ± 0.176	
	Rennebu	1,6	7,2	21,6	22,4	14,4	12,0	12,0	4,0	3,2	0,8	0,8						100		
	Ringebu																	100		
	Sum																	122	A ₄	
Twin-families in % of n.	Malsal	1	6	7	16	23	24	16	15	8	5	1						122	5.93 ± 0.189	
	Rennebu	0,8	4,9	5,7	13,1	18,9	19,7	13,0	12,3	6,6	4,2	0,8						100		
	Ringebu																	100		
	Sum																	100		

TABLE VI. Statistical survey of fertility (number of births per fraternity) in the whole Norwegian family-material (ser. 1), in twin-families (ser. 2), twin-lines (ser. 3) and in twin-fraternities (ser. 4). The percentage series 1* and 2* serve as a basis for the curves of Fig. 7.

Number
of
Frater-
nities

of follicles (see above, p. 129), might also prove its effect in the occurrence of a great number of single births.

This supposition is strongly supported, further, through the unanimous results reached in multiparous mammals¹ that "the frequency of production of litters" as well as "the average size of the litter" both increase with the age of the female, at least up to a certain point." (Jones and Rouse 1920, p. 265.)

Of special interest too is the evidence from various races of sheep², these animals forming a link between multiparous and uniparous organisms, in so far as twins and triplets are born in relatively high percentages besides their normally single births. The predisposition to multiple births is here known to vary characteristically from one race to another and to be hereditary within special lines. Here again we find an increase, with the age of the mother, of general fertility as well as of production of twins, and the question obviously arises whether these two phenomena (here referring to dizygotic twin-births only) should not be considered as different expressions of one and the same hereditary character.

This question was for our family-material raised in the preliminary report already (Bonnievie 1919), 380 fully investigated marriages from the Ringeby family being arranged according to the number of births in each of them, and compared with 96 marriages belonging to twin-lines. The average number of children was found to be somewhat higher in the latter (6.72 ± 0.25), than in the former (6.38 ± 0.14); but the whole material then at our disposal was too restricted, and the probable errors of the values found were, therefore, so high as to reduce very considerably the significance of this result.

Once again, therefore, the same question has been investigated, this time upon 1178 apparently full fraternities chosen without any selection from the whole *family-material* of Meldal, Renneby and Ringeby (Table VI, ser. 1), as compared with similar fraternities from special twin-families. These latter have been collected in three different ways, either (ser. 2) from whole *twin-families* comprising all their genetical lines with or without "twin-heredity," or (ser. 3) from "twin-lines" only, like those of the pedigrees on Pls. XV—XVII, or finally (ser. 4) through a selection of "twin-fraternities" chosen at random from the whole family-material. In each case some 125 fraternities have been used for investigating the fertility of twin-material.

The four groups of fraternities above mentioned have been arranged

¹ King (1917, 1924) in rats; Ellinger (1921) in swine.

² Rietz and Roberts (1915), Wriedt (1917, 1923), Roberts (1921).

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according to their number of births, twins always being counted as one birth only. As will be seen from Table VI the average fertility in the whole *family-material* proves to be 6.09 ± 0.065 , while in the "*twin-families*" it is 6.78 ± 0.222 and in the "*twin-lines*" 6.76 ± 0.176 . In both these cases the difference in the fertility between twin-families and the general population is worth noting, even if it can scarcely be called convincing, the differences of the average numbers of births amounting to, or to somewhat more than, three times their probable error. It should be remembered that the twin-lines are also included in the family-material, which may perhaps have helped to raise its fertility.

For a direct comparison of the fertility the distribution of fraternities has within each group of material been expressed also in percentage of

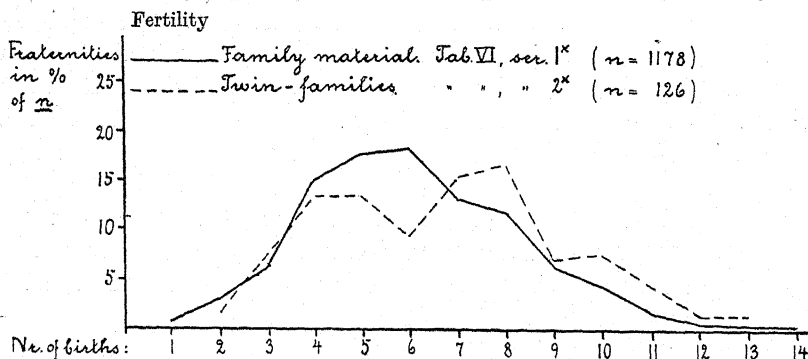


Fig. 6. Fertility, number of births per fraternity, within the whole family-material as compared with that of special twin-families (cf. Table VI, ser. 1* and 2*).

their sum totals (Table VI, ser. 1*–4*); the two curves of Fig. 6 constructed upon such series give a graphical illustration of the fertility of the whole family-material as compared with that of the twin-families.

It is not without interest that the rise of the average fertility found in *twin-families* as well as in *twin-lines* does not prove to be valid for the *twin-fraternities*. This may of course be due to the scantiness of material; but at any rate it proves that even if there may be some causal connection between high fertility and a predisposition for dizygotic twins, such connection does not exist in form of any linkage between two hereditary factors. If such had been the case the mothers of twin-fraternities ought to represent also the highest fertility.

In the following chapter, therefore, the predisposition for dizygotic twin-births may be investigated as an independently heritable character, its type of heredity being discussed on the basis of the actual occurrence of twin-births in our family-material.

CHAPTER III.

TYPE OF HEREDITY OF DIZYGOTIC TWIN-BIRTHS.

In the foregoing chapter we found the character hereditary in our family-material to be that of a *predisposition to dizygotic twin-births*, or in other words to a double ovulation, and consequently the *twin-mother* will be the person primarily interesting as carrying the hereditary factor. She, however, may have received this factor from any one of her parents or earlier ancestors whether males or females. Therefore, during the genealogical preparation of our work we have paid special attention to tracing the ancestry of every single twin-mother as far back as possible.

According to church-registers as well as to "Records of division of inherited property" a great number of families have been reconstructed up to the second half of the 17th century. These various families prove to be interrelated so as to form together a comparatively small number of very extensive pedigrees (from Meldal, Rennebu and Ringebu) all containing some 9-10 generations and amounting together to some 10,000 individuals¹ (see Table I, p. 42).

From such approximately complete pedigrees it has been possible for a considerable number of twin-mothers of later generations to trace their whole ancestry 5-6 generations backwards. For twin-mothers belonging to older generations the ancestry available is, of course, less complete, while in many cases, also, some branch of the family which has immigrated from other parts of the country has not been followed further back.

For each one of some 200 twin-pairs from Meldal and Rennebu tables of descent like that of Fig. 7 have been constructed, special stress having been laid upon tracing the ancestry of the twin-mothers. Besides this, the ancestry of about 80 twin-mothers from Ringebu has been thoroughly investigated.

In these tables the ancestry also of the twin-father will often be found when belonging to some of the families occurring on our pedigrees, but special investigations have as a rule not been made as to the ancestry of the twin-father.

As will be seen from Fig. 7, each table of descent comprises in all six ancestor-generations, beginning with the parents of the twin-pair, which means that space has been arranged for 126 ancestors, all generations taken together. To facilitate reference the numbering of these

¹ It is, of course, impossible to publish such pedigrees in full. They will therefore be kept in the "Institut for Arvelighetsforskning" of the University of Kristiania.

[illegible]

Fig. 7. Ancestry-scheme for a pair of twins from Meldal, on the right half of the fig. for the twin-mother. In the various spaces (1-126) of this scheme will be found the initials and the birth-years of ancestors together with their number on the pedigrees (in []) of Meldal or, if the figure is preceded by an R. (space 10, 21, 44), on those of Rennebu. In each space is found, further, the sex of the ancestor and, if he (or she) belongs to a twin-line, a mark expressing the degree of relationship with a twin-mother (cf. Table VII).

spaces has been made continuously through all generations beginning with the parents of the twins. In these schemes have been noted for each ancestor not only name and birth-year but also family-number in the pedigrees and several other data, as year of marriage, place of living, etc., which have been omitted in Fig. 7. Of importance for the question of heredity is the introduction into these schemes also of special symbols showing the relationship of an ancestor to other twin-mothers. An explanation of these symbols which will be found also on the pedigrees, is given in Table VII.

●	means twin-mother	
○	" twins	
□	" sister or brother of twin	
⊙	" sister or brother of twin-mother	
◻	" sister or brother of father or mother of twin-mother	
△	" son or daughter of sister or brother of twin-mother	
△	" son or daughter of a) twin b) sister or brother of twin	
△	" distant relation to twins	

TABLE VII. Survey of markings used on ancestry-schemes and on pedigrees to show the degree of relationship with a twin-mother.

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The symbols call attention to all individuals who, because of relationship with twin-mothers, might be suspected to be conductors of the hereditary factor. Without an exact knowledge of the type of heredity it will, however, be impossible to tell to what degree of relationship such probability should be extended.

In an investigation of the descent of a predisposition to dizygotic twin-births, considered as a character, three difficulties must be borne in mind.

(1) This character must be *sex-limited* in its phenotypical appearance. If carried by men, it may pass unperceived through several generations and then, perhaps, suddenly appear again in a woman belonging to an apparently free line.

This difficulty may be partially obviated by the supposition of an occurrence of men-conductors in a number equal to that of the women carrying the hereditary factor, the latter alone being counted and the final number of twin-mothers being doubled in order to give the supposed number of "twin-parents."

(2) The phenotypical proof of the existence of twinning-heredity does not, even in a woman, appear until the following generation. All women without children should, therefore be left out of consideration.

(3) A double ovulation seems, according to our experience, to be of a rather rare occurrence even in women carrying the hereditary predisposition for dizygotic twin-births, "repeated" twins occurring in only 7 per cent. of all twin-mothers of our material. Therefore, a woman who because of her heredity ought to be a "twin-mother" may perhaps not have manifested this character although she may have had several single births.

As a matter of fact, not only the childless women but also all those who have borne less than five children have been treated separately; this limitation is based on the official statistics of Norway (1916-17) that more than 70 per cent. of all twin-births (1216 out of 1709) occur among the first five pregnancies. The probability of a woman being a twin-mother without having shown it through her births, will thus be very considerably diminished by such exclusion of families with less than five children.

This restriction of the material considered is, of course, very severe, reducing the number of individuals to a small fraction only of those really born. But at the same time the restriction is in no way selective, and the results reached will, in spite of the reduced figures, gain very much in reliability.

The three restrictions mentioned must, however they are effected, all work in the same direction, that namely of reducing the actual occurrence of twin-births below the frequency expected according to the laws of heredity.

(a) *Sex-linked inheritance.*

The possibility of a causal relation between fertility and dizygotic twin-births raises the question of a possible parallel with Pearl's results. As shown by him (1915) the fertility in the common fowl is inherited partly as a *sex-linked* character.

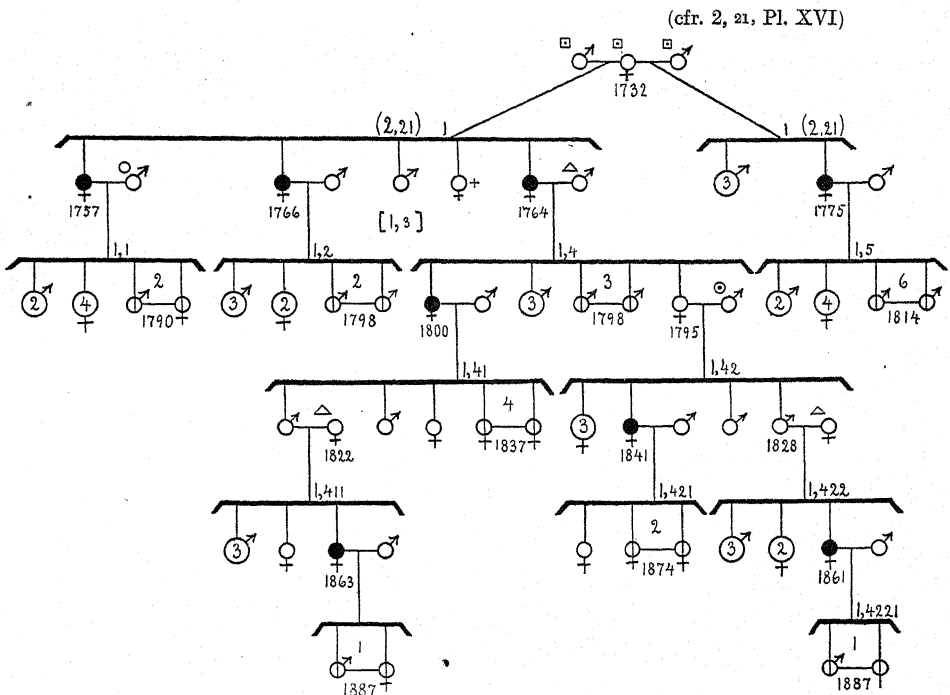


Fig. 8. Small fragment of a pedigree (see explanation to Pls. XV, XVI).

Even in our material small pedigrees may be found which, isolated, might seem to indicate that our character is a *sex-linked dominant*. Thus in the pedigree of Fig. 8 (a small part of the larger pedigree of Pl. XVI) we find in the first generation a woman, who has in her first marriage five, and in a second marriage four children: in all, four sons and five daughters, none of them being twins. This woman as well as

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both her husbands belong to twin-families all three having twin-mothers among their nieces.

On the supposition of a sex-linked inheritance, the predisposition for dizygotic twin-births should pass from a man with his sex-chromosome, to all his daughters, while none of his sons should become conductors.

In the pedigree of Fig. 8 this might seem to be the case: among the five daughters of the first generation one died young and all the others proved to be twin-mothers; the four sons have, on the other hand, no twin-mothers among their descendants. On the same assumption of a sex-linked inheritance the daughters should be expected to pass their predisposition to half of their daughters as well as to half of their sons. In fraternity 1, 4 (Fig. 8) one of the two daughters (1, 41) is a twin-mother and her son (1, 411) again a conductor, the other daughter has borne no twins. The twin-mother (1, 421) appearing among the children of the latter might have received a new inheritance from her father who is seen to be himself the brother of a twin-mother of another family. The only case, which in the pedigree of Fig. 8 would not fully agree with the supposition of sex-linked dominance is that of the twin-mother, no. 1, 4221. Her father should be supposed not to be a conductor, and if the inheritance had been carried by the mother, she ought herself to be a twin-mother, a possibility which is, however, not excluded even if she has so far borne no twins.

But pedigrees like that of Fig. 8 are mere exceptions and a review of the whole material makes it certain that the predisposition for dizygotic twin-births cannot be considered a dominant sex-linked character. The inadequacy of such hypothesis is, as an example, proved by the small pedigree of Fig. 9, taken from Pl. XVII.

In the first generation we have a fraternity of eight children, among whom there are no twins; the mother therefore is in all probability not a twin-mother. On the supposition of sex-linked dominance the heredity should therefore have been introduced by the father, and presumably carried on to all his daughters and to none of his sons. Only one of the daughters (2, 1) appears, however, to be a twin-mother although her two sisters have nine and five children respectively, while on the other hand, two sons appear as conductors, one of them (2, 2) having three, the other (2, 3) two twin-mothers among their children.

In case of a *recessive sex-linked* inheritance a woman should never become a twin-mother except when receiving the factor in question from both her parents, while a man should receive his inheritance from the mother only. A transference of the inheritance through

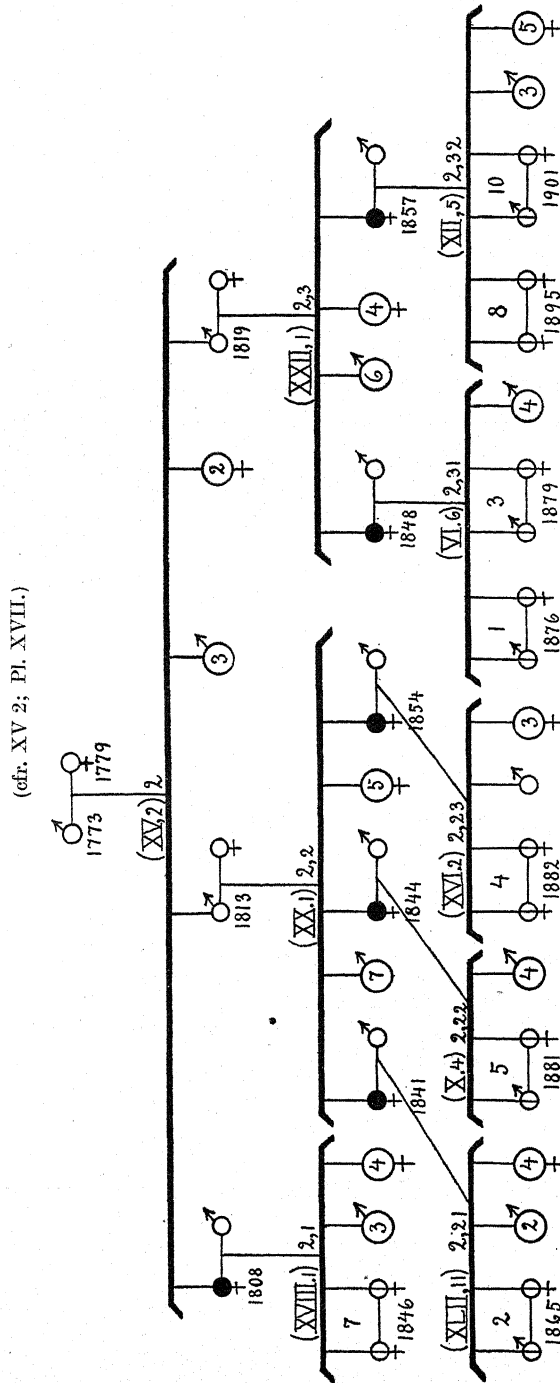


Fig. 9. Fragment of pedigree (see explanation to Pl. XVII).

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males in two consecutive generations would, thus, not agree with this supposition.

As a matter of fact it would, however, be impossible to establish sufficient proof for such relations. The conditions of the women would here be just the same as in case of pure recessivity and no sex-linkage can be proved by their inheritance only; and as for the men, the limitation of the phenotypical appearance of the character in question to the female sex makes it impossible to decide in each special case, whether they are carrying the hereditary factor or not.

(b) *Dominance.*

In case of pure dominance the hereditary character ought to appear in a series of successive generations. Acknowledging the difficulties, mentioned above, caused by the sex-limitation of the character in

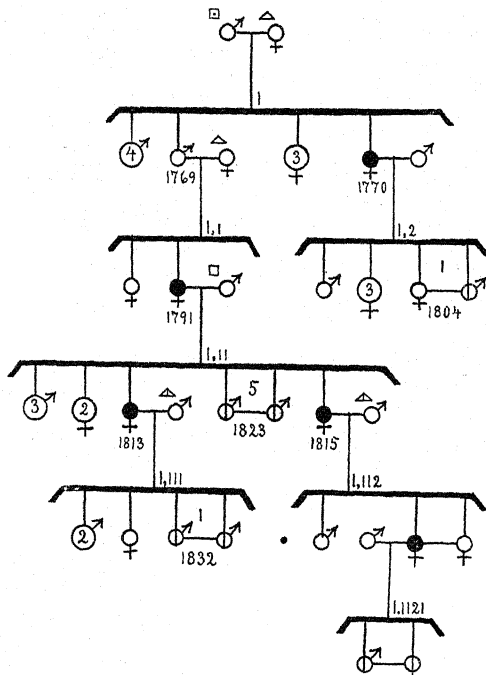


Fig. 10. Fragment of pedigree (see explanation to Pls. XV, XVI).

question, we should nevertheless expect to meet, frequently, with cases in which such transference is visible.

Looking over our large pedigrees we find, indeed, four or five cases,

in which twin-mothers appear in at least three successive generations. Examples of such inheritance will be found on Pl. XVI, from Rennebu; one case from Ringebu is reproduced also in Fig. 10.

We find here one twin-mother (no. 1, 11) who among her nine children has two daughters (1, 111 and 1, 112) proving to be twin-mothers; one of these has, again, a twin-mother (no. 1, 1121) among her children. The first mentioned twin-mother (1, 11) is further, daughter of a man (no. 1, 1) who is the brother of a twin-mother (no. 1, 2) and therefore, very probably, himself a conductor of the hereditary factor. Here we have, thus, no less than four successive generations displaying the character in question giving, if considered alone, a good illustration of pure dominance.

<i>Fraternalities including a twin-mother</i>				
	<i>born by a twin- mother</i>	<i>not by twin-m.</i>		<i>Sum total</i>
		<i>> 4 indiv.</i>	<i>< 5 indiv.</i>	
Meldal	12	42	40	94
Rennebu	15	43	38	96
Ringebu	8	29	11	48
Sum total	<u>35</u>	<u>114</u>	89	238

TABLE VIII. Statistics on the parents of 238 twin-mothers (see text).

Attention should, however, at once be drawn to the fact that at the same time, even in this pedigree, the necessary conditions are found also for a recessive inheritance, the parents of each twin-mother both belonging to twin-lines.

On the whole, and taking into consideration the large number of twin-mothers (more than 400) in the Norwegian family-material, the cases indicating pure dominance are remarkably few. But acknowledging on the other hand the many different reasons for a phenotypical suppression of this character (p. 158) as well as the possible existence of modifying factors suppressing the dominance phenotypically it will be

impossible, from the pedigrees alone, to reach any definite decision with regard to the question of dominance.

In order to advance a further step an investigation has therefore been undertaken with regard to the parents of twin-mothers (Table VIII), starting from the supposition that, in case of pure dominance, a twin-mother ought to have received her inheritance from each of her parents in the proportion 1 : 1.

As shown in Table VIII, 35 twin-mothers have, in the Norwegian pedigrees, been found to be themselves daughters of twin-mothers, thus having evidently received their inheritance from the mother. Among the other fraternities including twin-mothers, all those (89) have been left out of consideration in which the number of children is less than five (see above, p. 158). No less than 114 fraternities consisting of five or more individuals still remain in which a twin-mother is not seen to have received her inheritance from the mother, and where, therefore, the father should be supposed to be the conductor. But this proportion 35 : 114 is very far from the expected 1 : 1, so far indeed that this difference makes the assumption of a dominant heredity very improbable, allowance being made for all the above reservations.

(c) *Recessivity.*

On the assumption of recessivity each twin-mother should be considered a recessive homozygote, while both her parents must be conductors of the hereditary factor, that is either hetero- or recessive homozygotes. This condition has been investigated for all the twin-mothers occurring in our pedigrees, the ancestry of each being thoroughly studied. In order to estimate the hereditary qualities of the parents of a twin-mother we have found it necessary to know her ancestors for at least three generations with all obtainable information as to their genealogical relations, fraternities, descendants of sisters and brothers, etc. For many twin-mothers we could not, of course, obtain full knowledge.

The results of our investigation with regard to 176 twin-mothers from Meldal and Rennebu are given in Table IX.

A full knowledge of the ancestors, within at least three generations, has been reached for 46 twin-mothers all showing in the ancestry of both parents the presence of twin-heredity. 50 other twin-mothers belong to families well known on one side, either of their father (24 cases) or of their mother (26 cases) and here again, twin-heredity is without exception proved to exist on the well ascertained side of their ancestry,

and very often also on the other side. For 80 twin-mothers, finally, it has proved impossible to clear up three generations of their ancestry on either side; but even here twin-heredity has been found to exist in 19 cases upon both sides, and in 15 and 31 cases on the side of their father or their mother respectively, while in ten cases twin-births are found to occur within, or among the descendants of the fraternity of the twin-mother in question. Summing up, we find among 176 twin-mothers from

<u>Twin - Heredity</u>						
<u>Ancestry known in 3 gen. for:</u>	in ascendance of			in fraternity	None	Sum total
	both parents	father	mother			
	Both parents	46				
Father	14	10				24
Mother	7		19			26
None	19	15	31	10	5	80
	86	25	50	10	5	<u>176</u>

twin-mothers

twin-mothers

(from Meldal & Rennebu)

TABLE IX. Survey of ancestry of parents of 176 twin-mothers from Meldal and Rennebu.

Meldal and Rennebu no less than 86, both parents of whom belong to twin-lines, while for 75 others the same may be said with regard to the side of their ancestry which has been fully investigated. For five twin-mothers only, with very insufficiently known ancestry, nothing can be said with regard to the probability of their parents being heterozygous. Conspicuous illustrations of such twin-mother ancestry are given in Fig. 11 *a, b*, one from Meldal, the other from Rennebu.

To these results may be added also those from Ringebu (Bonnievie 1919) where, besides 21 twin-mothers with insufficiently known ancestry, "67 twin-mothers, the ascendants of whom are known through several generations on one (30 cases) or on both (37 cases) sides, are without exception shown to descend from twin-families through both parents or through the one of them which is known."

On the whole therefore our investigations have, so far as it has been possible to reach a sufficient knowledge of the ancestry, exhibited without exception the conditions necessary for a recessive heredity to exist in our family-material.

This result does not, of course, in itself prove the recessive heredity

of dizygotic twin-births. Considering the high degree of inbreeding in the population of Norwegian valleys it is to be expected that a double-sided heterozygosis might be found in any family, even outside the twin-lines. Since in the very large series of cases investigated, the whole evidence is consistent with the hypothesis that the capacity for dizygotic twin-births descends as a recessive, nothing incompatible with

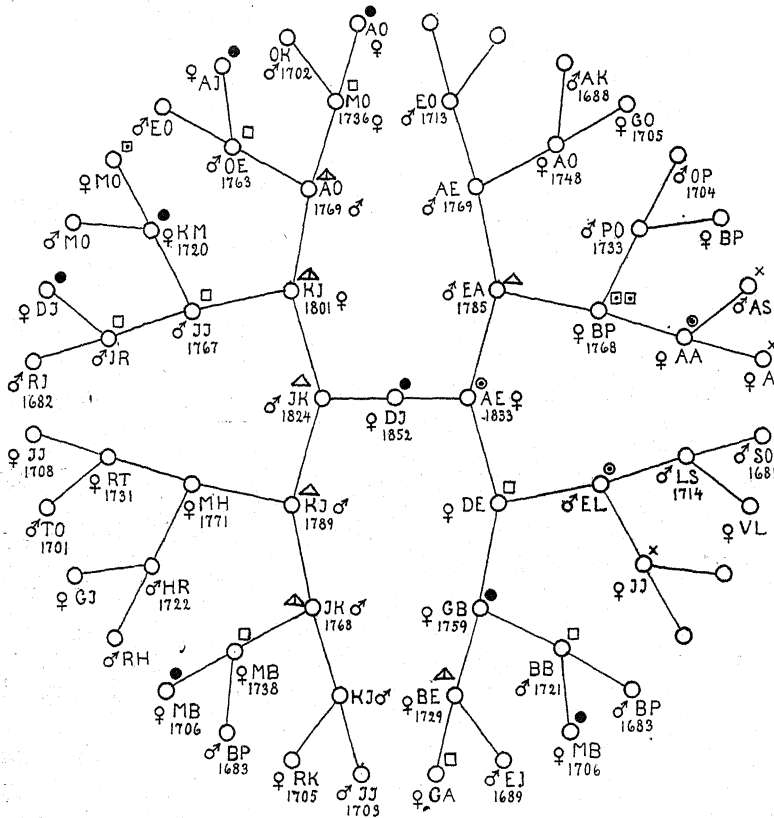


Fig. 11 *a*.

that representation having been seen (see p. 162), the conclusion must be regarded as well founded.

Our investigation with regard to recessivity should not, however, be finished without taking a survey also on the *genealogical* side of our material.

We very much regret the necessity of restricting our demonstration of this material to the examples found in the twin-lines of Pls. XV, XVI,

the twin-births here collected representing only a small fragment of those found on our full pedigrees. For saving space we have also been obliged to omit in these plates everything which was not of direct significance with regard to the genotypical relations of twin-mothers; all lines without any twin-births, having earlier been utilised for the statistical results, have therefore here been excluded. For comparison with our own

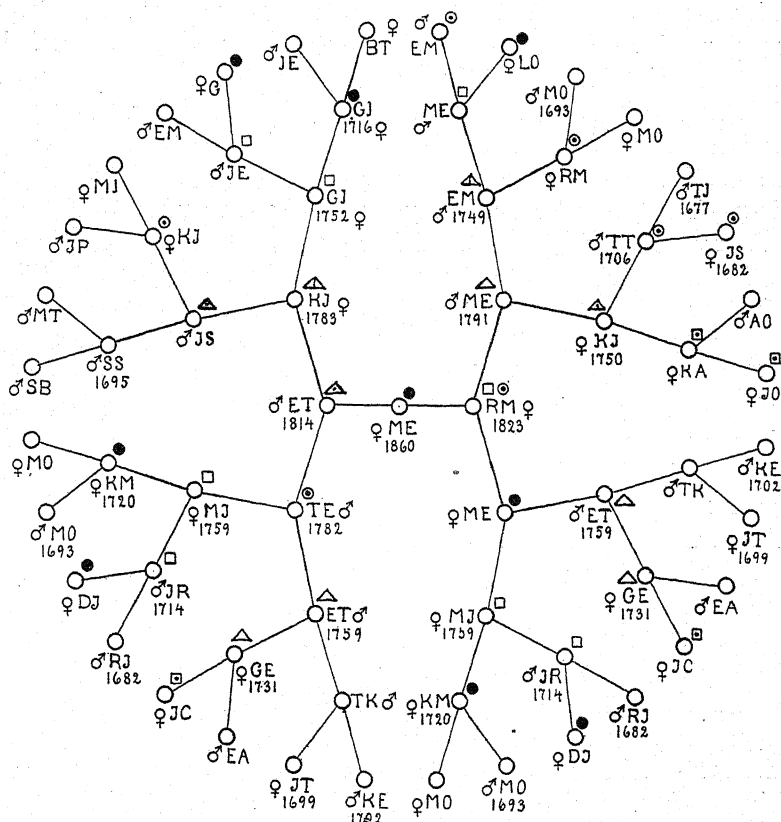


Fig. 11 b.

Fig. 11 a, b. Examples of ancestry-schemes, *a* from Meldal, *b* from Rennebu, showing twin-mothers (♀) always to descend from parents belonging to twin-lines.

material we have in Pl. XVII shown also the twin-lines of the large Blekinge family described by Lundborg (1913). Unlike the two other pedigrees Pl. XVII contains not only a fragment but the whole number of twin-mothers born within the large Blekinge family.

The three pedigrees of Pls. XV—XVII, representing each some specifically characteristic features, will in the following be considered separately.

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The Dombu family, Fam. 2, the twin-lines of which are represented on Pl. XV, is an old, and therefore also a widely spread, family within the parish of Meldal. It contains upon our full pedigrees 326 fraternities with in all 1196 births, including 34 twin-births, the percentage of twin-births thus making 2.8 per cent. or somewhat below the average of our whole twin-family material (3.25 per cent.). The "twin-lines" of this family, represented on Pl. XV, contains 96 fraternities with 425 births, among which 29 or 7.5 per cent. twin-births, a percentage which is, again, somewhat lower than that (8.23 per cent.) of the whole group of twin-lines considered on Table V (col. 7, line 9). Upon the whole, therefore, we have before us on Pl. XV a real twin-family, but at the same time a rather low-graded one. On the pedigree of Pl. XV the number of twin-mothers (29) amounts to 6.3 per cent. of all individuals (457).

A survey of the pedigree of Pl. XV proves first of all the result, already mentioned (p. 165), that whenever a twin-mother occurs, we find either both her parents or at all events the one of them whose ancestry is known, to have other twin-mothers among their relatives.

The first fraternity of this pedigree (2) does not include any twin-mother but it is, on the assumption of a recessive heredity, certain that at least one of the parents must have been a heterozygote, one of the daughters (2, 3) being the mother of a twin-mother (2, 32). Also in other lines (2, 1 and 2, 4) twin-mothers occur in early generations, making it probable that the same recessive heredity is present even here. In the line 2, 5, the twin-mothers are found only in later generations, and it would not seem impossible that in each of the three branches of this line (2, 51, 2, 52 and 2, 53) the twin-birth heredity had been introduced from outside. The fact however that twin-mothers are born in each of these three branches all descending from the same fraternity as 2, 3 and that, on the other hand, an introduction from outside to both parents of the twin-mother would need in each case at least two crossings with conductors from other branches, all this indicates a recessive heredity to be present also in the first ancestress (2, 5) of this family branch.

Upon the whole, the Dombu family gives an example of a typical twin-family with a relatively low number of homozygotes, as would be the case if, for example, in the first ancestor-pair, one heterozygote has been crossed with a dominant homozygote, and if the inbreeding with other twin-lines has not been very strong.

A different type of twin-family is represented in the Grindal family, Fam. 2 of Rennebu (Pl. XVI). The percentage of twin-births is here raised above the average to more than 10 per cent., more than 9 per cent.

of the 472 individuals of the pedigree being twin-mothers. In Pl. XVI *b* is shown the ancestry also of some individuals coming from other twin-families (within the "twin-lines" of this family) of the same, high-value type.

In these families we have before us not only several cases of twin-mothers occurring in a series of successive generations, but we find also a number of cases with two, or even 3-4, twin-mothers in the same fraternity. Pedigrees like this might at first sight make one think of a dominant type of heredity (see p. 162) but each separate case of twin-births fits at the same time equally well into a scheme of recessive heredity as working under a strong inbreeding between twin-lines.

The two types of twin families demonstrated in the pedigrees of Pls. XV, XVI are, in fact, good representatives of the whole population of the valley investigated: there are to be found large twin-families of the Dombu type (Pl. XV) with scattered strains of latent heredity for dizygotic twin-births carried through their various branches, but with no great number of twin-mothers, and there are branches in which the number of twin-mothers may be raised considerably above the average of all twin-families taken together, and which through inter-crossing may bring about results like that of the Grindal family (Pl. XVI *a-b*). The difference between these two types is, on the assumption of a recessive heredity, easily explained through variations in the genotypical starting-point of the family-branch in question, between the two extremes $DR \times DD$ and $RR \times RR$, and through variations also in the degree of inbreeding between twin-lines.

With regard to the Blekinge family (Pl. XVII) described by Lundborg (1913) we fully agree with this author when he says (p. 427): "Es ist augenscheinlich, dass eine etwas stärkere Anlage für Zwillingsgeburten seit älteren Zeiten innerhalb dieses Geschlechtes nicht existiert, denn da sollten wir eine weitaus grössere Anzahl Zwillinge innerhalb desselben erwarten dürfen, zumal die Verwandtenehen sehr gewöhnlich sind. Es sieht deshalb aus, als ob die Anlage für Zwillingsgeburten erst verhältnismässig spät dem Geschlechte zugeführt worden sei."

In Lundborg's admirably full and extensive pedigrees we find "das grosse Geschlecht" arranged in "Linie I—VI," each line descending from one of six sisters and brothers, borne 1745-64 by the first ancestors. Line V, which had already, 1799, left the parish, has not been worked out. To this large family, including 1909 births, of which 1.466 per cent. are twin-births, are added a few small pedigrees of Lundborg's, one or more individuals of which are married into the "large" family.

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With regard to the distribution of 28 twin- (and 1 triple-) births, Lundborg finds "dass Zwillingsgeburten in den verschiedenen Linien, wenn man deren Grösse berücksichtigt ziemlich gleichmässig verteilt vorkommen." (Table XXIV, p. 428.)

In order to compare and control our own results with regard to the heredity of twin-births we have ventured to rearrange the pedigrees of Lundborg so as to trace the ancestry of each twin-mother as far back as possible.

Such reconstruction is found on Pl. XVII, in which to facilitate reference control each fraternity carries, instead of the usual decimal-number, the number of the plate and family used by Lundborg in his atlas. It should be mentioned also that only such twin-mothers have been considered by us whose birth is to be found in the pedigrees.

From the pedigree on Pl. XVII it is evident that no less than half of the 24 twin-mothers considered may genotypically be included in the two small pedigrees of "Eskil Svensson" (Lundborg I, a 14) and of "Fam. B" which are seen to have, in directly descending lines, eight and four twin-mothers respectively. Among the six lines of the "large family" lines V and VI have given no proof of twin-birth heredity, while at the same time in line I, III and IV some hereditary factor for twin-births must have existed from rather early generations. It might, for example, under the assumption of a recessive heredity, be supposed that, in line I the twin-birth heredity has been introduced through the male parent of fam. 2, 1, in line III through the female of fam. 15, 1, and in line IV through the male of fam. 23, 1. Even in line II hereditary twin-factors must exist in order to explain the occurrence of the three twin-mothers in fam. 20, 1 (line IV); the heredity might here perhaps have been introduced through the male parent of fam. 9, 1. None of the persons mentioned are with regard to their genealogical relations known well enough to justify any conclusion as to whether or not they belong to twin-families.

The same result might, however, have been reached also through the supposition of a recessive heredity existing already from the beginning, in one of the first parents of the "large family," and spreading in the first generation to at least four of the six children (lines I—IV); with regard to line V we know, as already mentioned, very little.

If, after all, we think Lundborg right in his conclusion that the "large family" cannot be considered a twin-family, it is because of the large number of family-branches (not represented on Pl. XVII) in which no twin-heredity has shown its existence. Even in a very low-grade "twin-family" starting from a cross $DR \times DD$, we should expect a

spreading in the various family-branches far more conspicuous than that here found. The mere statistical result of no more than 1.466 per cent. twin-births, will in comparison with the 3.25 per cent. in the Norwegian twin-families, in itself be sufficient to prove that the occurrence of hereditary twin-births in the Blekinge family must be supposed to be restricted to certain branches only. This family thus supplements the two types of twin-families represented on Pl. I and Pl. II, the three pedigrees finding, in all their diversity, a full explanation in the assumption of a recessive heredity for dizygotic twin-births.

ANNEX: DO DIZYGOTIC TWIN-BIRTHS ALWAYS REPRESENT ONE
AND THE SAME CHARACTER?

As mentioned in chapter I of this paper (p. 135) we have, besides this family material, considered also an isolated case of such predisposition, a woman from Elverum who with her eight twin-births occupies an exceptional position among all our twin-mothers. Special stress has been laid, therefore, upon investigating the question whether or not we have before us, in this case, a hereditary character of the same nature as that of the other twin-mothers in our material.

Fig. 12 gives our present knowledge about the births and the family-relations of this woman. As will be seen from the pedigree the first four pairs of twins were born within the lapse of less than 3 years (24/4 1905—27/2 1908); then follow, within the next $4\frac{1}{2}$ years (until 18/9 1912) four single births, and then again at a somewhat slower rate during $6\frac{1}{2}$ years (until 29/3 1919) four new twin-births, and, finally, after $1\frac{1}{2}$ year a single birth. This youngest child is, when this is written, nearly 4 years old; the age of the mother is, however, no more than 44 years, and the number of births may perhaps still be augmented. At any rate she has, so far, borne 21 children within the lapse of $15\frac{1}{2}$ years. Ten of these children died during their first year, all the twins having, as told by the midwife, been born about one month too early, and the mother being, from the first birth on, absolutely incapable of suckling her children.

Four of the twin-pairs are bisexual, the remaining four pairs unisexual, but according to the midwife two chorions have been present in all twin-births. We have therefore, no doubt, before us a case of some anomaly in the ovaries making a double ovulation the rule instead of an exception, probably due either to a simultaneous bursting of two follicles or to the existence of follicles containing more than one egg. Ovaries showing both these kinds of anomaly have, as mentioned in the intro-

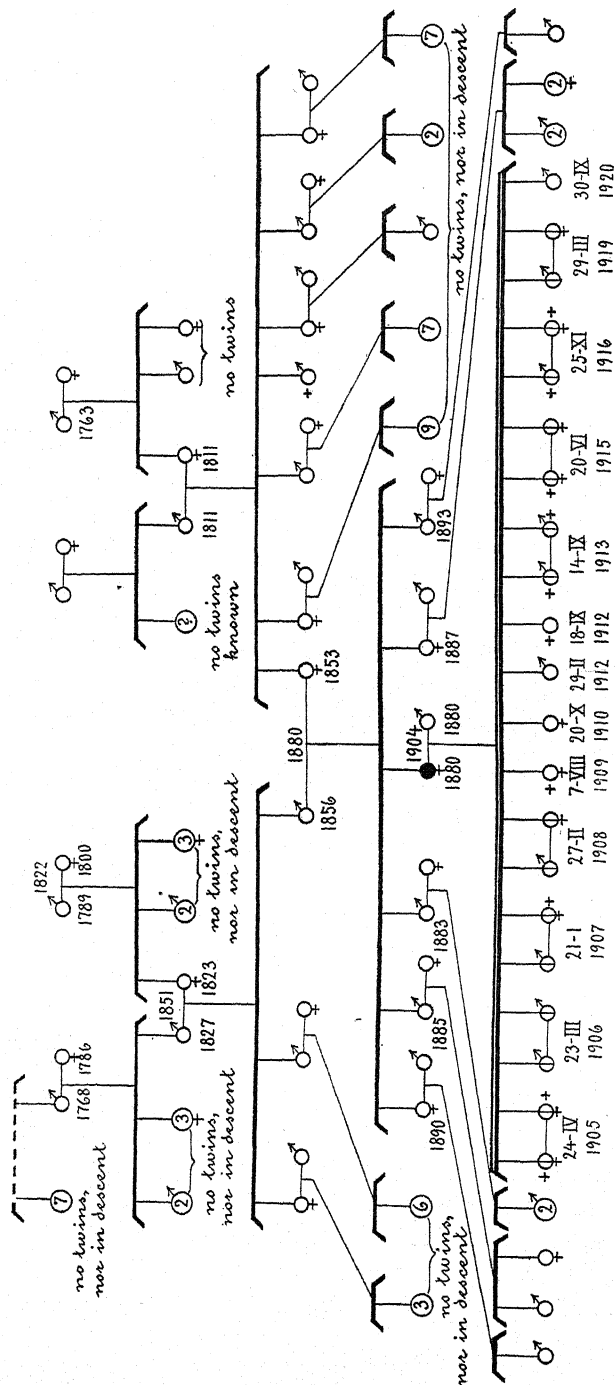


Fig. 12. Pedigree from Elverum showing the sudden appearance of excessive multiparity in a family where, otherwise, no twin-birth is known to have occurred.

duction (p. 128), been demonstrated already by previous authors. The lack of milk-secretion may perhaps also be seen in a causal connection with the anomalous ovary.

The question whether this character can be considered transmissible has, of course, been of special interest to the authors of this paper. Investigations have been made in the field as well as in the church-books, but so far with an absolutely negative result; no twins have been found in the family, and none of the twins occurring within the last century, in two parishes, have been found to descend from any known ancestor of the twin-mother in question.

The value of this negative result is not absolute, in so far as no more than three ancestor-generations have been investigated and, especially on the side of the mother of the twin-mother, with no satisfactory result. Possibly in some other parish twin-mothers might be found descending from the great grand-parents of this twin-mother.

But even so, the pedigree before us (Fig. 12) makes it very little probable that the predisposition for twin-births should in this case have been inherited. A dominant heredity ought certainly to have become apparent within several of the fraternities (some large) of the pedigree. In case of a recessive heredity, on the other hand, both parents of the twin-mother should be considered heterozygotes, an assumption which may, as already mentioned, perhaps not be excluded for her mother, though for her father that seems very improbable. He descends from ancestors living through generations within the parish of Elverum and even if the genealogical investigation has not been carried further back than the second half of the 18th century, the fact that none of the later twin-births of this parish can be traced back to any of the known fraternities makes it practically certain that the father of our twin-mother does not belong to any twin-family and that he is himself no heterozygote.

Upon the whole, therefore, it seems that we have before us in this woman a *first appearance* of an anomaly in the ovary causing, as a rule, double ovulations. The question whether this anomaly should be considered as hereditary cannot be decided until one or more new generations may be added to our pedigree.

Similar cases from cattle (Pearl 1912) as well as from man (Stocks (1861), Strassmann (1889), Rosenfeld (1903), Brattström (1917), Davenport (1919)), have, however, occasionally been described in literature, or even in newspapers; and in so far as these descriptions may be considered reliable, the anomaly seems to be inheritable.

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Special mention should be made of the one case only, which has been described by Davenport (1919). Here we find in a family from Cleveland one woman, three times married, with in all 15 multiple births, out of which 7 were twin-births, 5 triple and 3 quadruple births; no birth was single. "Thus the propoita has averaged nearly three children at a birth and has had thirty pregnancies inside of twelve years in the last mating, in addition to the eleven by the two earlier matings."

This very extraordinary case is evidently of the same di-(poly-)zygotic nature as the one described in this paper. In the two foregoing generations "only twins, triplets and quadruplets" were said to have been born, and even in the latest generation one twin-birth has occurred. The hereditary nature of the anomaly seems, therefore, in this case evident, and the whole pedigree gives a strong indication of a dominant type of heredity.

The whole appearance of such anomalous predisposition to multiple births makes us incline to consider it a character different from and independent of the more common hereditary tendency to dizygotic twin-births.

CHAPTER IV.

DISCUSSION.

We have now to consider how far our conclusions are in agreement with the results of previous authors. Looking over the genealogical investigations on twin-heredity we shall, as already mentioned in the introduction, find many of these pedigrees more or less incomplete, in so far as very often the ancestry was imperfectly investigated. From such incomplete pedigrees no reliable conclusions can, of course, be drawn. The most complete pedigrees on twin-heredity found in literature are those of Meyer (1916).

With regard to a comparison between pedigrees from different populations, it ought, however, to be remembered that we do not, *a priori*, know whether the twin-births considered are always comparable. When in our material, we felt justified in considering the hereditary predisposition to be to dizygotic twin-births, this view has been based upon the thorough statistical investigations of chapter II. Similar investigations will, indeed, be necessary also for each new twin-population before deciding the nature of the twin-births.

Thus in the large pedigree of Meyer (1916, Taf. IV) of princely families the number of unisexual twin-births (29) make more than three times that of bisexual ones (9), that is, we should according to the

difference-method have here at least as many monozygotic twin-births as dizygotic ones. This result may, of course, be due to mere chance, the number of twin-births here considered being too small to give reliable results. It shows, however, that care should be taken not to draw general conclusions from inadequate family-material.

An apparent discrepancy in our own preliminary results (Bonnevie, 1919) to which attention was drawn by Dahlberg (1923) arose through the scarcity of the twin-material then investigated. Dahlberg has pointed out, that in the Norwegian Ringeby family there seemed to be an augmentation not only of dizygotic, but also of monozygotic twin-births, when compared with the frequency of twins within the whole population. Statistical investigations upon our whole family-material (Table V, p. 59 of this paper) have, however, with regard to this question given the following results:

The frequencies normal to the Norwegian population (*Official Statistics of Norway*, 1916-17) are found in column 1 of Table V. The percentage of twin- (multiple-) births as compared with the total number of births, is 1.46 (line 9), while, according to the difference-method of Weinberg, 27.53 per cent. of all twin-births should be considered as monozygotic (line 19) and 72.47 per cent. as dizygotic (line 22). In other words 0.40 per cent. of all births are supposed to be monozygotic twin-births (line 20), while dizygotic twin-births seem to occur with a frequency of 1.06 per cent. of all births (line 23).

In column 2, giving the figures regarding the whole population (1750-1895) of the valley in which our new investigations have been made (Meldal-Rennebu (Fig. 1)), we find the general frequency of twin-births to be somewhat lower, 1.24 per cent. (line 9) than the norm given by the whole population, but the relation between the frequencies of mono- and dizygotic births is somewhat the same, viz. 0.36 per cent. and 0.88 per cent. of all births (lines 20, 23).

Corresponding figures for the family-material are found, for each family alone in cols. 3-5, and for all families together in col. 6, while in col. 7 the figures are given for special twin-lines of the families investigated.

The widely varying results reached for each separate family (cols. 3-5) through the difference-method will not surprise us if we remember that such calculation can be reliable only when based upon large figures (cf. also Table II), which is not the case for each family or for the twin-lines, found in line 8 of Table V. By summing up the results of all the families (col. 6), the influence of chance is, however, largely reduced,

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and here we find monozygotic twin-births in 0.47 per cent. of all births, a result which is seen to be very near that of the whole Norwegian population. Even here the number of twin-births is too low to be accepted as a definite result; it does not, however, suggest an increase in the number of monozygotic twin-births in our family-material. The whole increase (line 9) from the normal percentage of twin-births (1.46 per cent.) to that of the twin-families (3.25 per cent.) is, on the contrary, seen to be due to the corresponding increase of the percentage of dizygotic births (line 23) from 1.06 per cent. to 2.78 per cent.

When in our investigations upon the heredity of dizygotic twin-births the twin-fathers have been left out of consideration we are, as already mentioned, in full agreement with previous authors both clinical and gynaecological.

In a few recent papers upon heredity of twin-births the view has, however, been maintained that the twin-father might play a more or less important part not only in the production of mono-, but even of dizygotic twin-births.

Thus Meyer (1916) after having (p. 299) declared with regard to dizygotic twin-births that "diese Störung in der Funktion des Ovariums natürlich nur dem weiblichen Organismus selbst innewohnen (kann)" finds himself "auf Grund des Stammbaum-materials" obliged "auch diesen Vorbehalt als unsicher und fraglich bezeichnen zu müssen."—"Gewiss ist es uns," he continues, "bei den zur Verfügung stehenden Theorien der Entstehung Zweieiiger nicht leicht, uns eine rechte Vorstellung für direkte väterliche Beteiligung zu machen, aber bei Musterung der Stammbäume drängen sich einem geradezu eine Reihe von Fällen auf, bei denen die Nichtmitwirkung der selbst deutlich belasteten Väter von Zwillingspärchen, also sicher zweieiigen, einem als eine gezwungene Auslegung erscheinen möchte." The author then points out no less than ten different cases in his pedigrees which, according to his opinion, necessitate the conclusion that the twin-fathers acted as conductors. After careful consideration of all these cases, we do not, however, agree with his conclusion.

In the first case mentioned (Stammbaum VII, 62) both parents belong to twin-families, the mother being a sister of another twin-mother, twins existing also among the sisters and brothers of her mother, and her grandmother being herself a twin. In this case the fact that also the father belongs to a twin-family should not be sufficient to "necessitate" any conclusion with regard to his influence on the two twin-births among his own children.

A similar case, both parents belonging to twin-families, is represented also in Stb.VIII, 160.

In six other cases, mentioned by Meyer, the family of the mother is either not at all, or at any rate only imperfectly known (Stb.VII, 69, 70; Stb.VIII, 151, 198, 199, 224).

There remain still two crossings (Stb.VIII, 111 + 112) in both of which the same man, belonging to a twin-family, is the twin-father.

Ringebu. Fam. 3

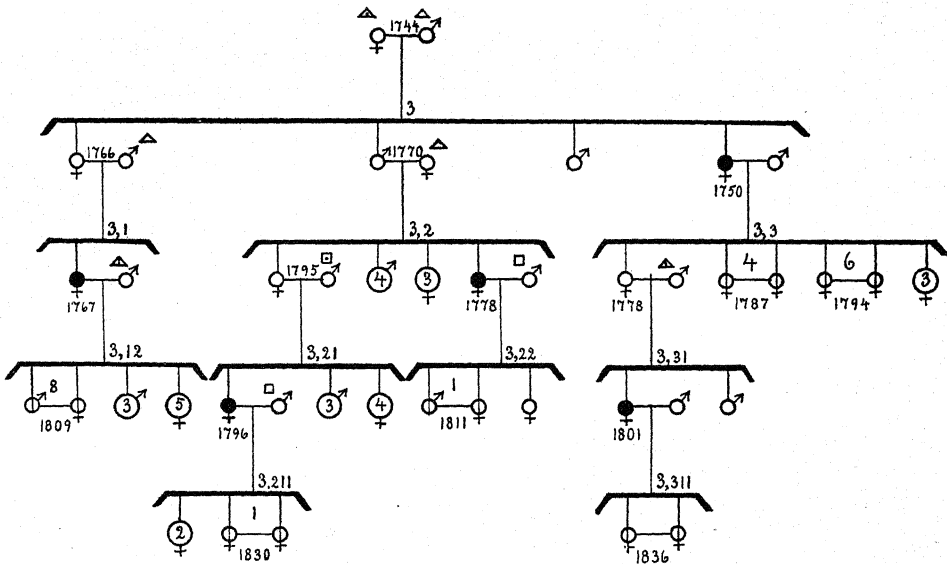


Fig. 13. Fragment of pedigree from Ringebu (cf. Table VII, and explanation to Pls. XV, XVI).

Here again, however, the ancestry of the twin-mothers is in one case absolutely unknown, in the other known only on the side of her father, while no information is given with regard to her mother.

None of the ten cases, therefore, quoted in support of Meyer's view, appear to us as being in any way conclusive in favour of a hereditary influence of the twin-father upon dizygotic twin-births. We find in our own family-material a whole series of cases in which not only the mother but also the father of twins belong to twin-families. If, for instance, in the pedigree of Fig. 13 the ancestry of the twin-mothers had not been known, we should have had before us no less than three families (3, 12;

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3, 22; 3, 211) in which twin-births might seem to have been caused by the fathers. With full knowledge also of the ancestry of the twin-mothers, we find, however, in all these cases in the family of the mothers alone a fully sufficient basis for the hereditary appearance of twins, each of the five twin-mothers of this pedigree being born of parents both belonging to twin-families.

Also Davenport (1920) draws, from his statistics upon a material of "repeater-families" (with more than one pair of twins), the conclusion that (p. 125) "the father has about as much influence in the production of twins as the mother."

This material of Davenport has, as it seems, been collected by field-workers, and the "repeaters" belong to a great many different families. No distinction has been made between mono- and dizygotic twin-births; no information is given either with regard to a possible relationship between both parents, or with regard to the number of cases in which not only the fathers but the mothers also of "repeated" twins belong to twin-families.

It is clear, therefore, that the frequency-figures given in the paper of Davenport cannot *prove* anything with regard to the conductors of the tendency to mono- or dizygotic twin-births. Even if it must be acknowledged that the statistics of Davenport represent a surprisingly high number of cases in which the fathers of "repeated" twins belong themselves to twin-families, we fully agree with Weinberg (1924) who maintains that the material of Davenport is small in comparison with his own, which proves beyond doubt the overwhelming hereditary influence of the twin-mothers.

Davenport (1920 *a*) sets forth also an hypothesis to explain how the father may have an influence upon the production of twins (dizygotic). "It may well be," he says (p. 125) "that two eggs are simultaneously ovulated much more frequently than at present recognized and that the comparative rarity of twin-births is due either to a failure of fertilization of one egg, or to a failure of development of one egg."

That such "failures" of fertilization or of development really exist he concludes from an investigation of "birth-intervals" within a certain generation of "the Gorton family." Assuming the normal interval between births to be about two years, he looks upon "the frequent intervals of 3, 4, 5 and even more years" as examples of "failures," although he admits the possibility also of other explanations. In analogy with the results reached upon certain animals, he thinks the failures due to "lethal factors," representing (p. 128) "widespread phenomena even

in human germ cells," and accounting "for a certain proportion of long intervals between births, of early miscarriages, and of sterile unions."

According to this view he looks upon (p. 128) "twins that are born" as "the residuum of a greater number of twins that start in their development, and of a still greater number of pairs of eggs simultaneously ovulated," reaching at last the conclusion (p. 129) "that among humans the cases of twin-repeating fraternities are those in which there are no or few lethal factors in the germ cells, so that there is a maximum fertilization and development of the eggs laid"—and further "that families which easily produce twins do so not only because in the mother the eggs were laid in pairs, but also because in the father the sperm is active, abundant and without lethal factors, so that the number of eggs fertilized and brought to full term approaches a maximum."

The question here touched by Davenport about the numerical relations between *actual* and *observed* production of twins is one of great interest, but at the same time one which will not easily be definitely solved. At this place we shall refer to it only in so far as his hypothesis might raise an objection to the view here taken.

The reasonings of Davenport seem to be based upon two different assumptions, viz.:

- (1) that a double ovulation is a common occurrence in man, and
- (2) that the relatively rare occurrence of twin-births, as well as a natural occurrence of long birth-intervals, is due to lethal factors in one or both germ-cells causing (a) a failure of fertilization, or (b) a failure of development of the embryo.

With regard to the first assumption about the frequency of double ovulations a thorough investigation of the literature, anatomical as well as gynaecological, proves the ovulation of single eggs to be typical for the human ovary. Descriptions of double and multiple ovulations may be found (see Arnold 1912); but such examples are always considered as exceptions from the general rule.

But, on the other hand, it is very probable that the *actual* production of twins may, even in man, be considerably larger than that *observed*—one or both twin-embryos being checked in their development at an early stage (Neugebauer 1913).

Hammond (1914) in his discussion of the cause of atrophic foetuses in swine comes to the conclusion, that (p. 273) "possibly the atrophy of the embryo may be caused by reduced vitality due to something inherent in the foetus, and this may often be augmented by inbreeding."

This leads us to the second assumption of Davenport as to lethal

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factors causing (a) a failure of fertilization, or (b) a failure of development—the latter part of which has found a support already in the results of Neugebauer and Hammond.

A failure of fertilization, as caused by lethal factors in the sperms may of course also occur, leading to more or less complete sterility of certain crossings. But in his hypothesis Davenport seems to consider the failure of fertilization of an egg a common occurrence in free breeding, and upon this point our experience from Norwegian peasant-families does not support his view.

In order to test the *birth-intervals* in such families, the number of years during which the five first births have occurred has been noted for one whole generation of our Ringeby family, comprising 91 fraternities with 5–10 or even more children. Here no less than 58 mothers, or 63·7 per cent., have borne five children within 11 years, an average birth-interval of two years, while further 16 mothers or in all 74 (81·3 per cent.), have borne their fifth child before the end of the 13th year—and even this last lapse of time gives no great space for “failures of fertilization.” In 86 other fraternities, chosen at random from the church-books, the corresponding figures are not much lower, viz. 55·8 per cent. with five births within 11 years, and 77·9 per cent. with five births within 13 years. Taking into consideration also the many different causes, physical and psychical, which may serve to augment the birth-intervals of a human family, a failure of fertilization does not seem to be any common feature in free breeding.

But if a double ovulation from the human ovary represents an exception from the general rule, and if the abundance and activity of the sperm should normally be considered sufficient for fertilization of the eggs present in the tube, then the explanation given by Davenport for the part played by the father in twin-production has lost its principal base, and does, therefore, not raise any objection to our considering the *twin-mother* as being alone responsible for the production of dizygotic twins.

A thorough investigation of the whole literature concerning twin-births has, indeed, revealed to us no facts opposed to the views maintained in this paper, nor to the assumption reached that in the Norwegian peasant families investigated a predisposition for dizygotic twin-births is inherited as a, probably monohybrid, recessive character, while at the same time exceptional cases of an excessive multiparity may occur which do not follow this general law of heredity.

SUMMARY.

(1) The results of this investigation have been based upon a genealogical, as well as on a statistical, treatment of a series of Norwegian peasant families inhabiting two neighbouring parishes, Meldal and Rennebu, near Trondhjemsfjord, and the parish of Ringeby in Gudbrandsdalen. The pedigrees of these families include some 10,000 births among which are some 430 twin-births¹. Use has been made also of the twin-branches of the Swedish Blekinge family described by Lundborg (1913).

For a comparison, and for extension of our statistical results, we have used the material of the *Official Statistics of Norway* for 1916-17, as well as of that of the "Women's Hospital" of Kristiania for 1816-1916, including 1848 and 537 twin-births¹ respectively (see Table I, p. 42).

(2) Within the populations of the parishes investigated twin-births have been found not equally scattered but accumulated on certain families and within these on certain twin-lines. While the percentage of twin-births in the Norwegian population proved to be 1.46, the twin-families gave 3.25 per cent. and in special twin-lines the percentage was raised even to 8.23 (see Table V, p. 59). Such accumulation of twin-births in special genetical lines is in itself a strong indication of heredity.

(3) Before investigating the type of heredity, the question had to be solved about the nature of the twin-births before us. The numbers of mono- and dizygotic twin-births calculated according to Weinberg's difference-method, and arranged according to the age of the twin-mother, have proved beyond doubt that the character inheritable in these families is that of a *predisposition for dizygotic twin-births*, or in other words, for a double ovulation from the ovary of the twin-mother (see Tables III, IV and Figs. 2-6).

(4) Investigations have been made also with regard to a possible causal connection between twin-births and general fertility. Although fertility seems to be somewhat higher within twin-families than in the whole population, no evidence has been found indicating the twin-mothers to be at the same time the women with the highest fertility (Table VI, Fig. 7).

(5) The type of heredity has been investigated through a thorough study of the genealogical relations of all twin-mothers, the twin-relations also of each ancestor being pointed out through special markings on the pedigrees and on the ancestry-schemes (Figs. 8-11, Table VII).

¹ Still-born included.

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(6) Although in exceptional cases, on small parts of our pedigrees, the occurrence of twin-mothers might be explained as due to a dominant sex-linked inheritance (Fig. 9), and although some restricted parts of our pedigrees might seem to indicate a pure dominant type of heredity (Fig. 11 *a, b*) such assumptions find no support in the numerical results (Table VIII).

(7) As for recessivity—twin-mothers being themselves recessive homozygotes—the necessary conditions: that both her parents should be conductors of the hereditary factor, have so far as our results reach, been found to be present in our family-material (Table IX).

(8) In the pedigrees of Pls. XV, XVI, in which only the "twin-lines" have been included, examples are given of low- and of high-value twin-families, which may result from various combinations ranging between $DR \times DD$ and $RR \times RR$. Pl. XVII gives, after a rearrangement of the pedigrees of Lundborg (1913), some twin-branches introduced into the Blekinge family which is probably, in itself, no twin-family.

(9) As an addition, has been investigated also a separate case of excessive predisposition for twin-births, a woman from Elverum having borne no less than 8 pairs of twins. No twin-births were found among her relatives and it seems very improbable that her parents should, both, have been heterozygotes. The question whether this anomaly should be considered as hereditary cannot be decided until new generations are known.

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EXPLANATION OF PLATES XV—XVII.

PLs. XV, XVI. Pedigrees of twin-families, Fam. 2 from Meldal (Pl. XV) and Fam. 2 from Rennebu (Pl. XVI).

Only the "twin-lines" (genetical lines leading to twin-births) are demonstrated on these pedigrees, while for individuals not entering into these lines, the posterity has been omitted. The full pedigrees are kept in "Institut for Arvelighetsforskning" of the University, Kristiania.

The decimal-system used for fraternities and individuals will scarcely need any explanation.

All twin-mothers are on the pedigrees marked with black, while for their parents and earlier ancestors markings of relationship with other twin-mothers have been used as explained on Table VII, p. 157.

A note of interrogation means that the person in question belongs to an unknown family, or that his ancestry has not been cleared up for so much as three generations.

Years of birth have, as a rule, been noted for twins and for twin-mothers only; the figures added to all twin-pairs, above the line connecting the twins, means the number of each twin-birth in the series of births of the fraternity.

Figures in () added, obliquely, to some persons married into the family means the number of the fraternity to which the person belongs. Such figures have not been added except when this fraternity belongs to one of the twin-families of Pls. XV and XVI.

When on Pl. XVI (2, 111 and 2, 1121) reference is made to M. 2, 3213 and M. 2, 5313, this means the Meldal family, Pl. XV.

Some names of the principal farms on which the twin-families have been, or are, living will be found added to special branches of the families.

To the Rennebu family of Pl. XVI *a*, an annex (Pl. XVI *b*) has been added, as containing the ancestry of several persons married into the family mentioned.

PL. XVII. Pedigree showing the twin-branches of the Blekinge family, based upon a rearrangement of the very extensive original pedigrees of Lundborg (1913).

As in Pls. XV, XVI all descent from persons not of genetical interest for twin-births has been left out.

The usual decimal-designation of fraternities has on this pedigree been omitted in order to facilitate control of our rearrangement—for the designations used by Lundborg himself; XLV 3 means, for instance, fam. 3 on Pl. XLV of Lundborg's Atlas.

Even here the markings of relationship used on Pls. XV, XVI and explained on Table VII, p. 65, have in a few cases been introduced on the pedigree.

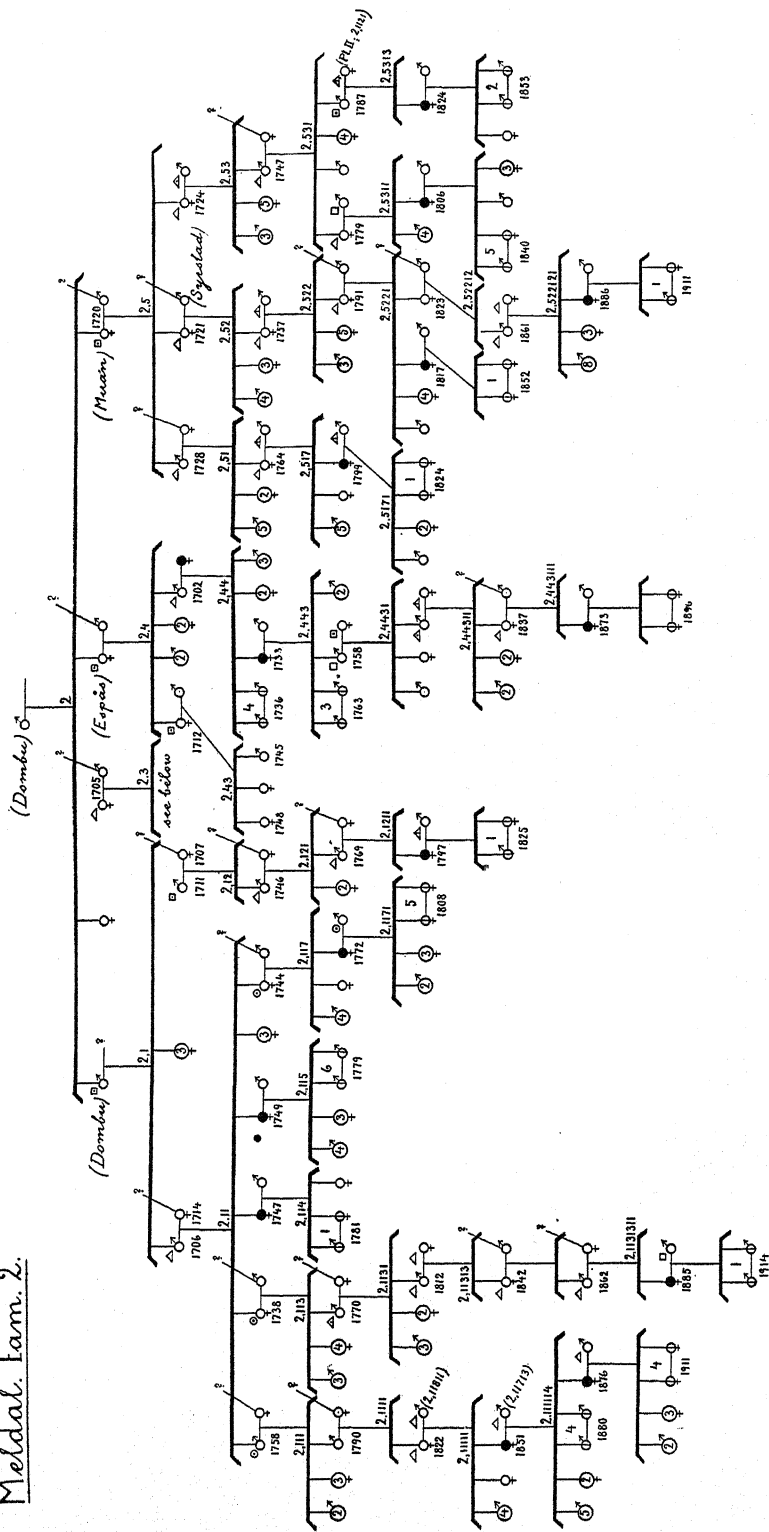
Material		Age of mother:		17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	?																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																								
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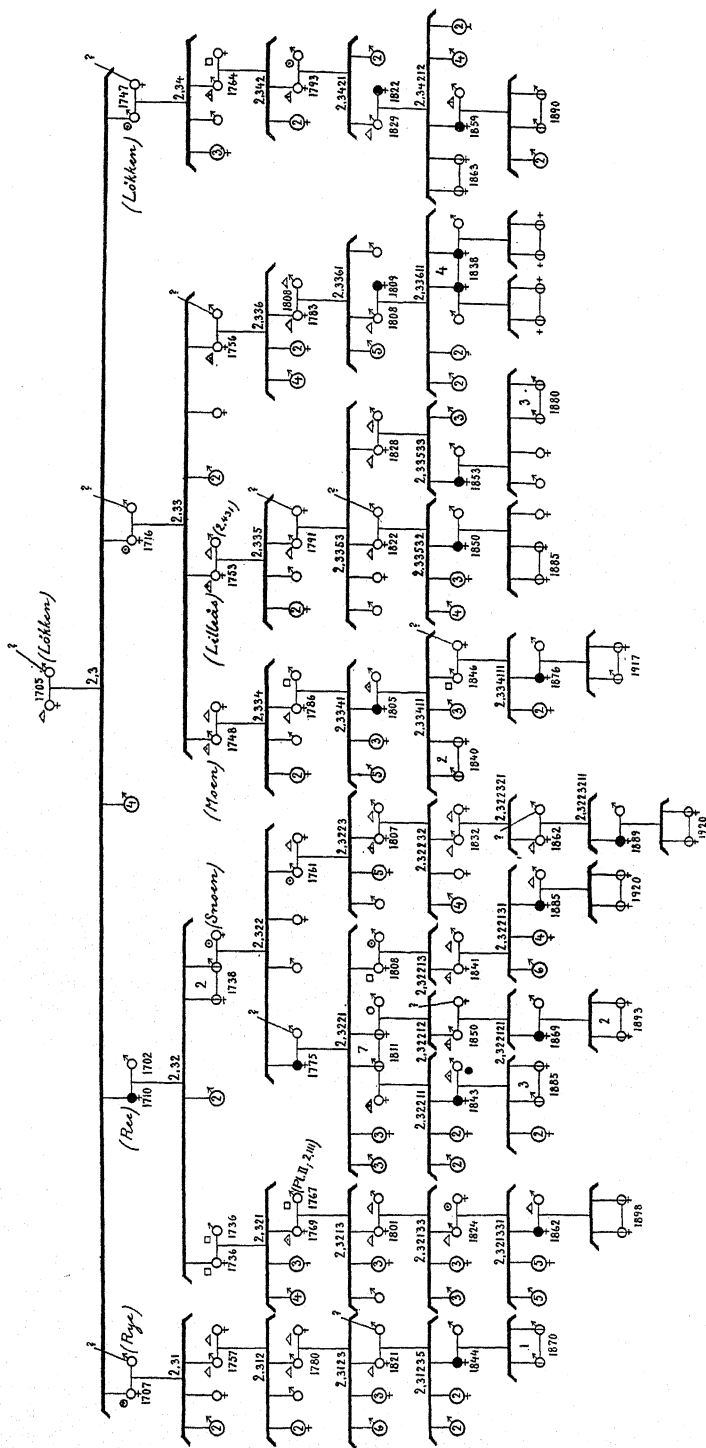
[illegible]

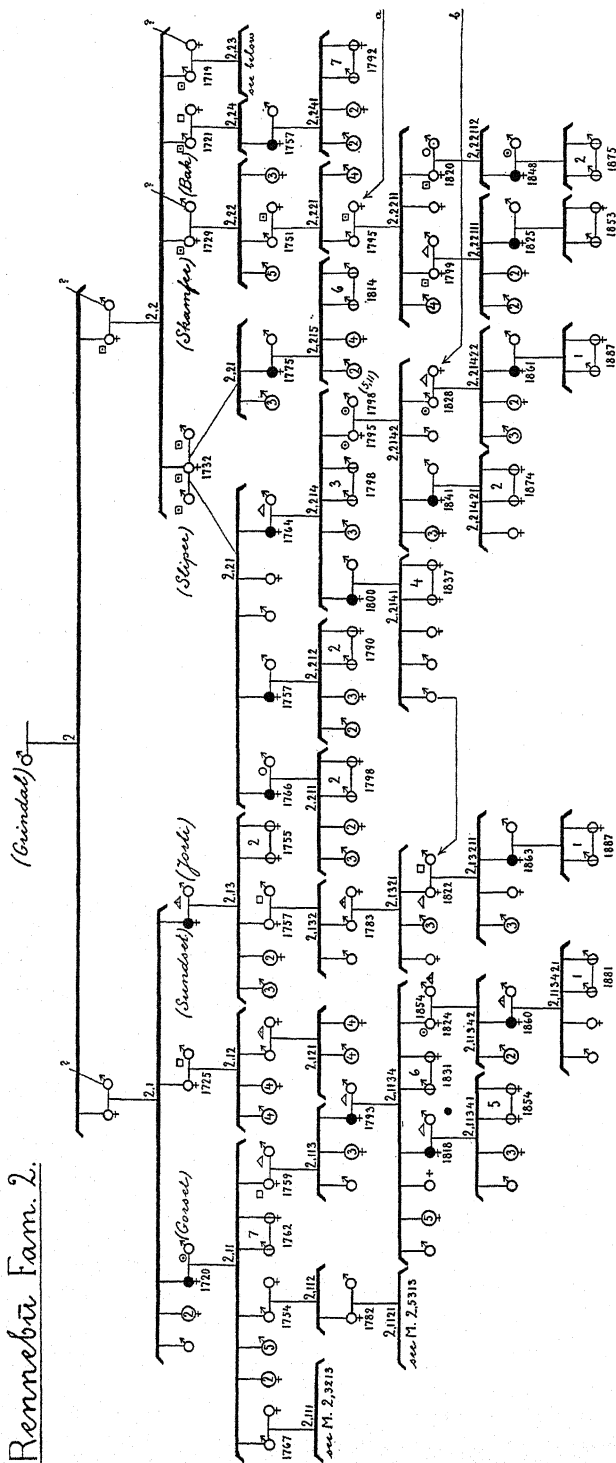
TABLE III b.

TABLE III *a, b*. Statistical analysis of twin-births from the various groups of material considered in this paper, arranged according to the age of the mothers and the sex of each twin-pair. The numbers of mono- and dizygotic twin-pairs have been calculated by the difference-method of Weinberg. For comparison, and as a supplement, the twin-births of the Blekinge family described by Lundborg (1913) have also been added to the statistics of the Norwegian peasant families.

Meldal. Fam. 2.





Rennetbär Fam. 2.

(Stegau) ♂
1719

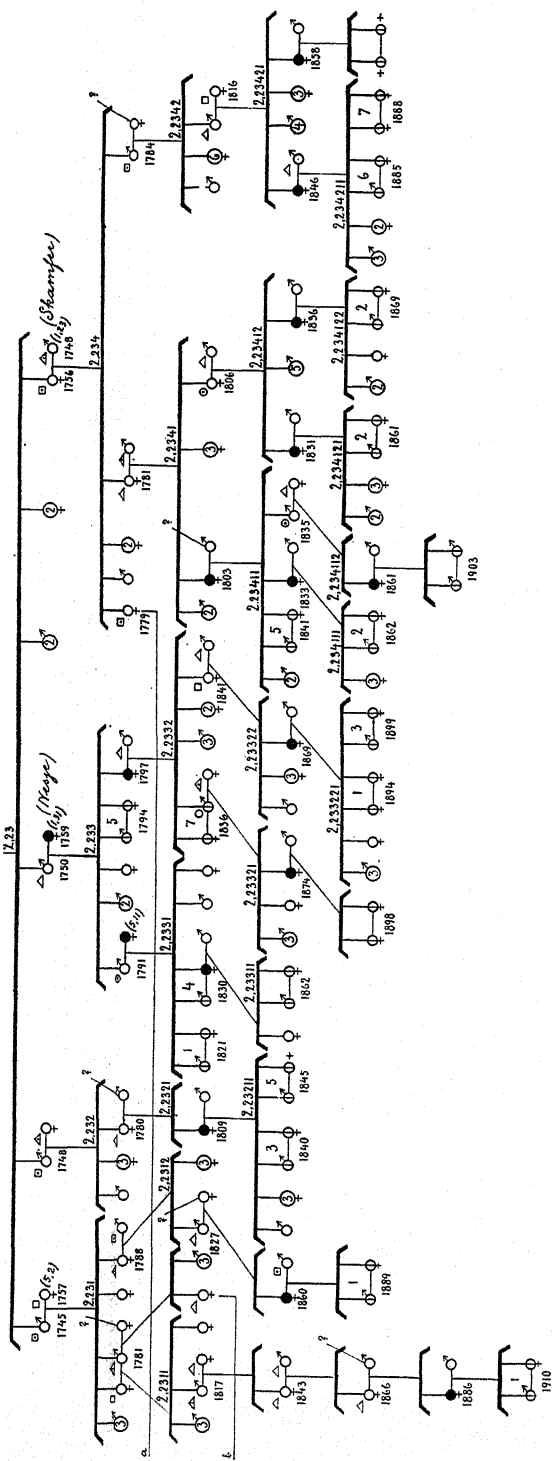
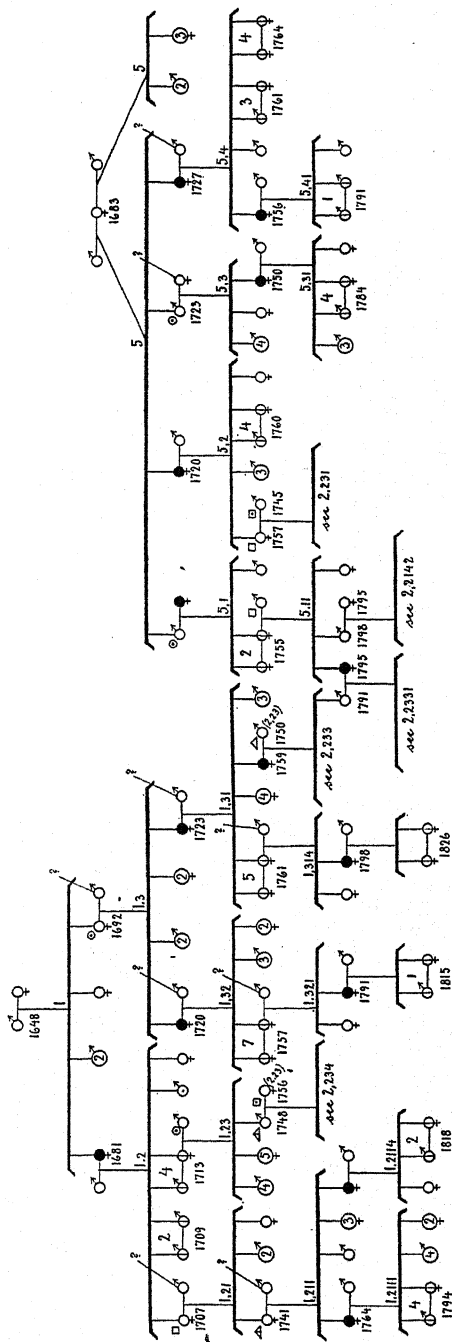


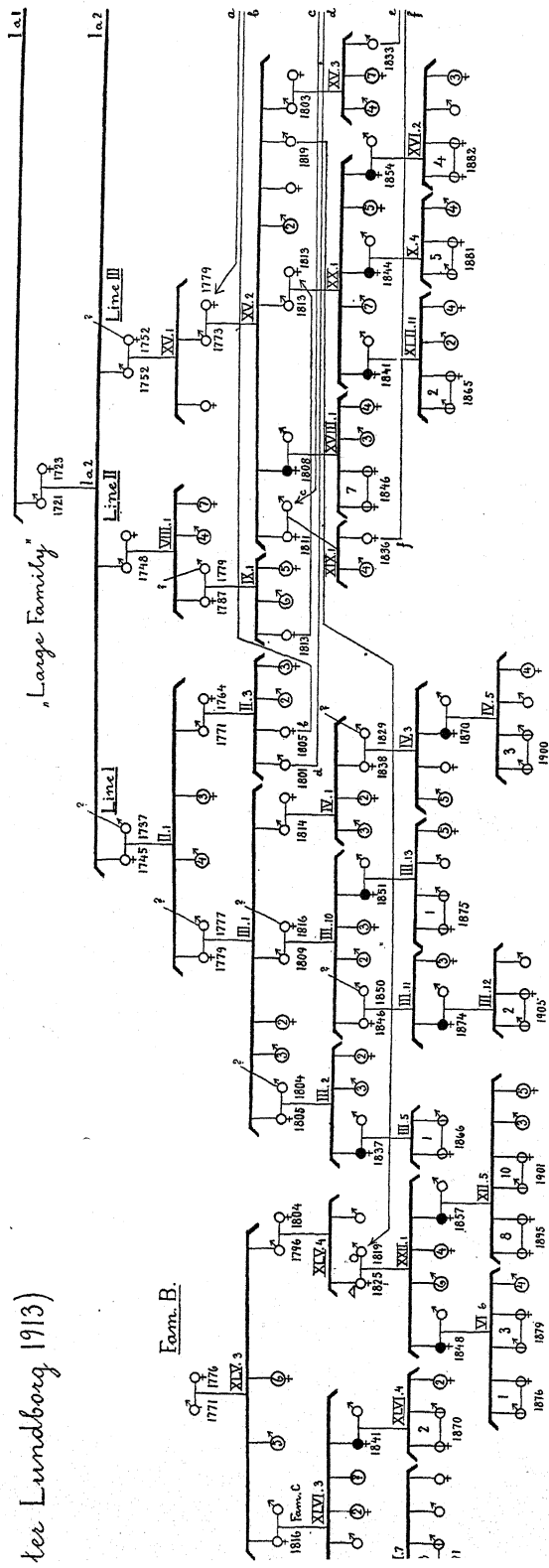
PLATE XVI b

Rennebiu Fam. 2 (Annex.)

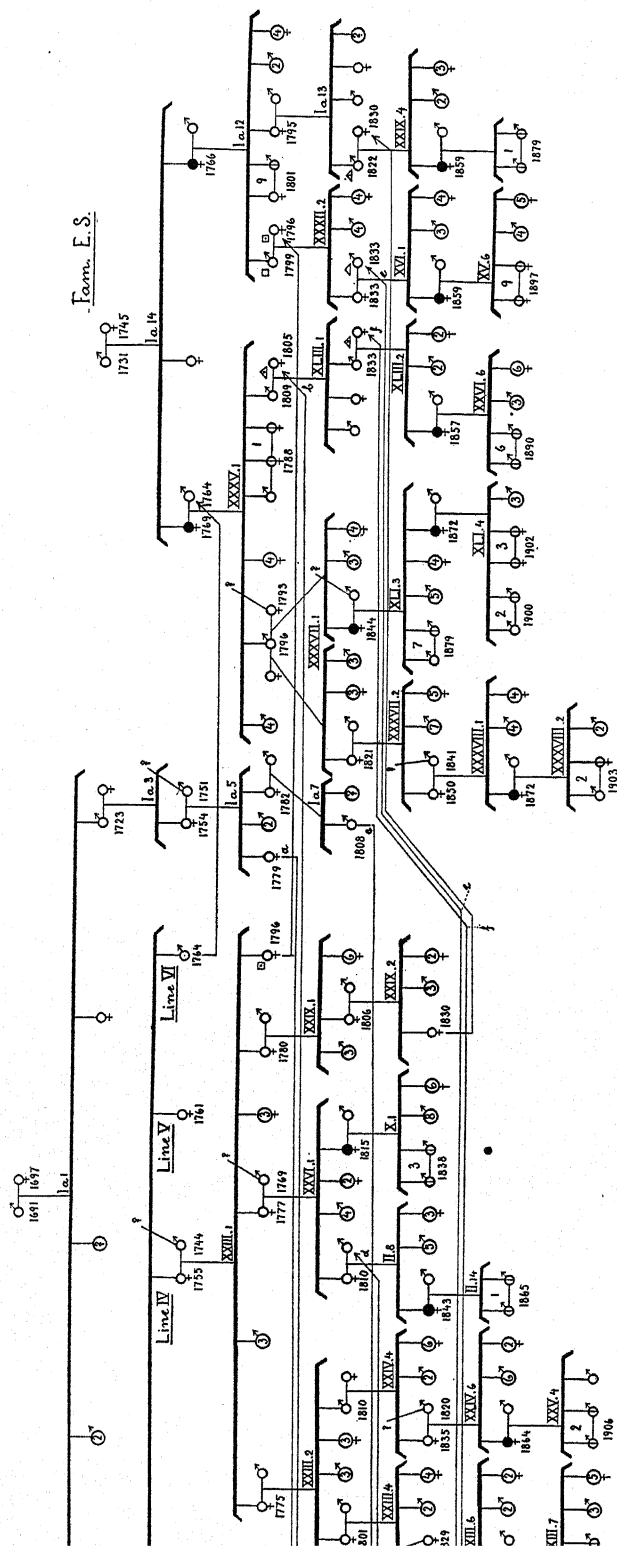


inge-family, Twin-lines.

ter Lundborg 1913)



Form. E.S.



ON THE PATTERN OF THE DUTCH RABBIT, A DISCUSSION OF THE RESULTS OF PUNNETT AND PEASE.

By W. E. CASTLE.

(With Two Text-figures.)

GENETIC studies of the Dutch rabbit have been made by a number of investigators, more particularly in recent years by Professor Punnett and myself. Our findings as to fact agree in a remarkable way but our interpretations are quite divergent, at least they seem so to a reader of the recent paper of Punnett and Pease¹. In reality they are not so far apart as they seem.

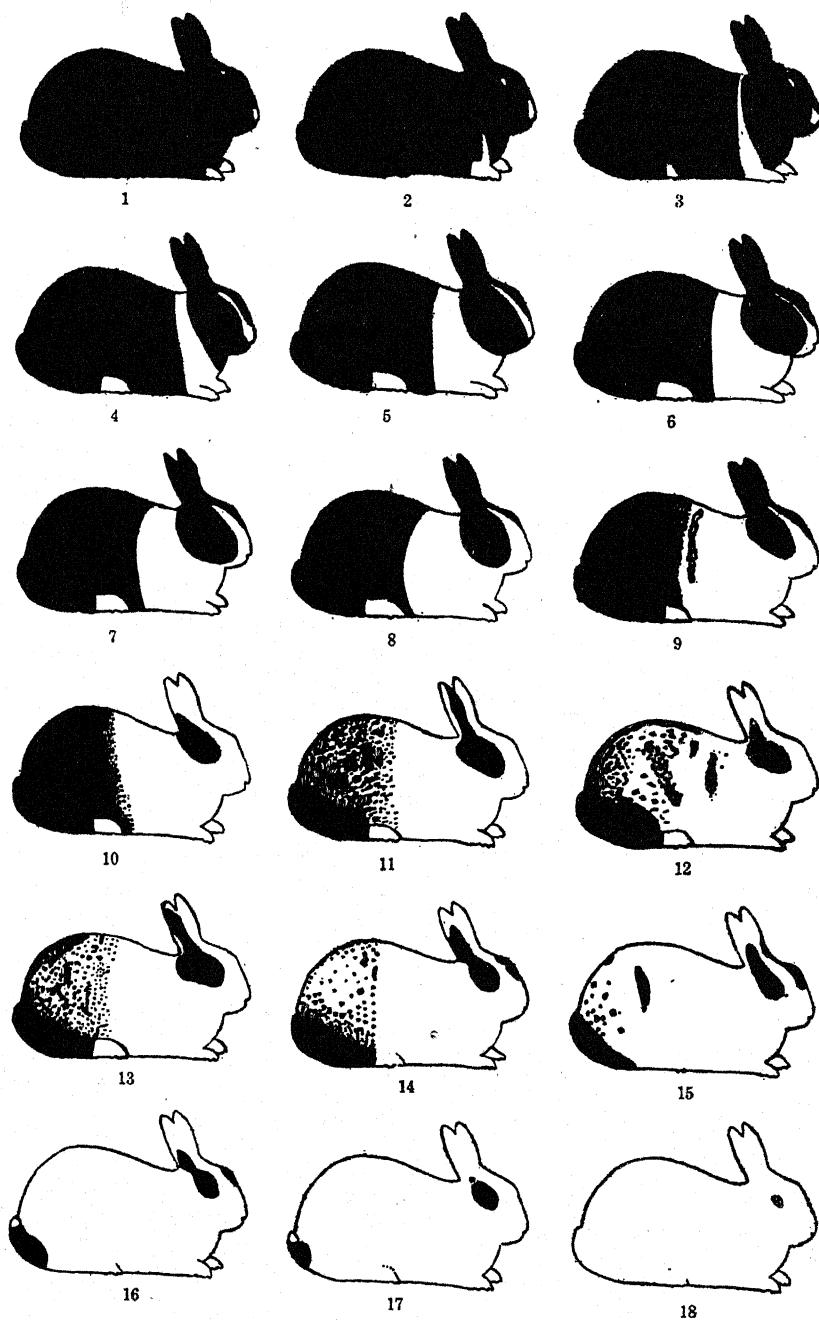
In 1919 I advanced the view that variation in the Dutch pattern (which is extraordinarily great) was due to two agencies, (1) variation in the Dutch gene itself, which assumes several allelomorphic forms, and (2) the modifying action of genes other than the Dutch gene proper, "residual heredity" as I then designated it. Punnett (1920) attacked the first of these conclusions vigorously in a review of my paper published in this *Journal*, and Punnett and Pease continue irreconcilable to the idea of multiple allelomorphs in the case of Dutch pattern, though meantime Punnett has adopted it in the case of the Japanese rabbit².

Punnett offered as a substitute for the idea of allelomorphic conditions of a single Dutch gene, an explanation which would make Dutch pattern dependent on the combined action of three independent genes, *P*, *S*, and *T*. Punnett and Pease now find it convenient to invoke the existence of a fourth "definite" Dutch factor, *N*, in addition to certain supposed modifying factors, *X* and *Y*.

It would seem to be a very easy matter to reach a decision between rival hypotheses, one of which called for monohybrid inheritance ratios, the other for trihybrid or tetrahybrid ratios. In reality Punnett and Pease have not made the direct experiment of crossing their supposed multiple recessive variety (White Dutch) with the supposed multiple dominant (self). It may be of interest therefore to inquire what the results of such a cross are and whether they suggest a tetrahybrid or a monohybrid segregation among the gametes formed by F_1 individuals.

¹ *Journal of Genetics*, Vol. xv. p. 375, July, 1925.

² *Ibid.* Vol. xiv. August, 1924.



Grades 1-18 of Dutch rabbits.

Fig. 1.

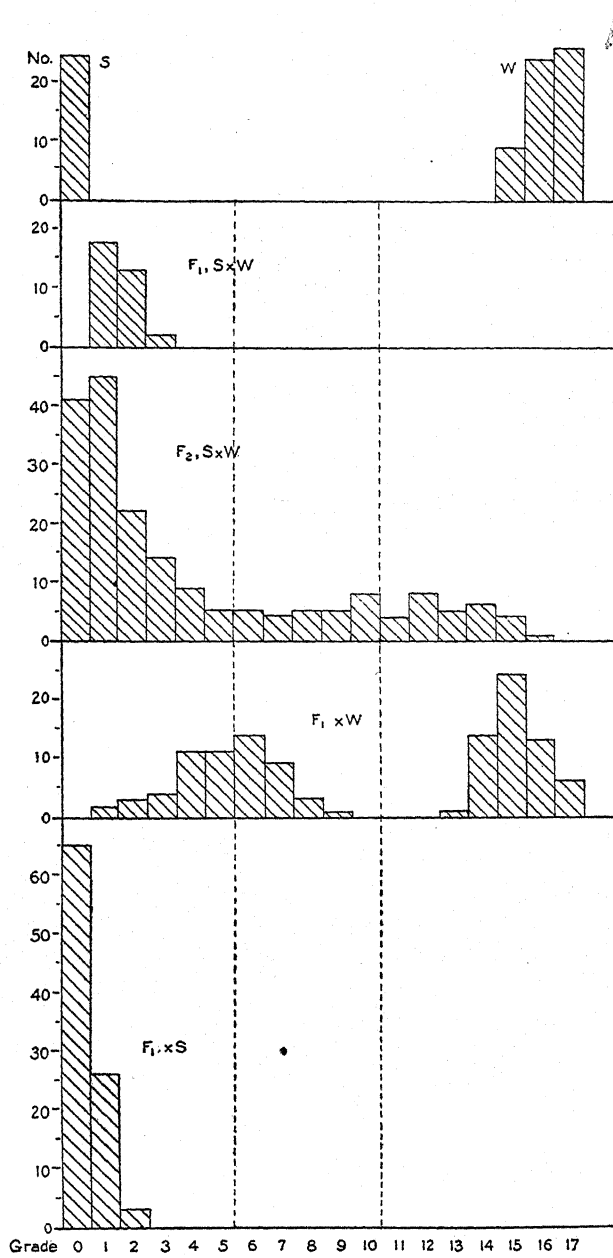


Fig. 2.

This cross I made several years ago and reported on its results in considerable detail in 1919. The results are presented graphically in Fig. 2, in terms of the grades shown in Fig. 1. The range of variation in the race of White Dutch (W) used in making the cross, is shown in the top row of Fig. 2 as from grade 15 to 17, while the self race (S) was true breeding (grade 0). The grade of the F_1 young is shown immediately below ($F_1, S \times W$), ranging from grade 1 to 3. The F_2 generation ranged from grade 0 to grade 16. The back-cross with the white race ($F_1 \times W$) is particularly instructive. It shows two (and only two) distinct, monomodal groups, containing 58 individuals each. It would be difficult to imagine a clearer demonstration of unifactorial segregation. I so interpreted it in 1919 and so interpret it now. The hypothesis of Punnett and Pease, on the other hand, suggests a difference of four major factors between self and White Dutch, the former being $N P S T$, the latter $n p s t$. It is difficult to see how a four-factor hypothesis can be reconciled with the obvious 1 : 1 segregation of the back-cross to the White Dutch parental variety. Nevertheless Punnett did, in 1920, attempt to fit a three-factor scheme to these very figures. Doubtless with a sufficient number of subsidiary hypotheses, making certain factors inactive when their presence would otherwise be troublesome, the fitting can be done as well with four factors as with three.

If, in crossing White Dutch with self in the foregoing experiment, one uses Dutch individuals which have long hair (Angora) and self individuals which have short hair, the F_1 young all have short hair, Angora being a recessive character. When the back-cross is then made with the Dutch Angora race, approximately half the young are short-haired and half long-haired, obviously a unifactorial segregation for the Angora character as well as for the Dutch. But the long-haired segregates are mostly White Dutch, as the long-haired were exclusively in the P_1 generation, and the short-haired segregates are mostly like the F_1 generation in grade. In a particular experiment of this sort, which will be reported in greater detail elsewhere, there were produced 123 back-cross young grouped as follows:

$S W$ group (like F_1 in grade)	$W W$ group (like White Dutch)
54 short-haired	11 short-haired
9 Angora	49 Angora
Totals 63	60

There is unmistakable linkage here between White Dutch and Angora, and between self and short hair. An idea of the strength of the linkage may be gained by comparing the size of the *crossover* or recombination

classes with that of the *non-crossover* or original combination classes. The recombination classes number 9 and 11, the original combination classes number 54 and 49. In this experiment and others of a similar nature, a total of 1213 back-cross young has been produced, of which 173 only are recombinations or crossovers. The percentage of crossovers is accordingly 14.26 ± 0.96 . On the chromosome theory, this means that the Angora gene lies in the same chromosome as the White Dutch gene at a distance of about 14 units (morgans) from it.

When by similar methods an investigation is made of the linkage relation of Angora to Dutch in a race which I have called Dark Dutch, and which corresponds closely to what Punnett and Pease call Deep Dutch, it is found that here also linkage exists between Angora and Dutch. In 1919 I reported that White Dutch and Dark Dutch are allelomorphs. The fact that both show linkage with Angora supports this conclusion. Punnett and Pease now report that White Dutch and Deep Dutch contain different allelomorphs of the same Dutch factor, P , which is in their view the chief or basal factor for Dutch, the only one which, unaided by other factors, can produce a Dutch pattern. This is in complete accord with my findings in the case of White and Dark Dutch. We are agreed, then, that the Dutch factor proper (P) occurs in two different allelomorphic forms in two different true-breeding races of rabbits, White and Dark (Deep). I assume further that self contains a third allelomorph, and am able to show that it has the same linkage relation to Angora that the Dutch allelomorphs proper have. If Punnett and Pease were to admit that a *third* allelomorph of P exists in self races, they would be fully committed to a multiple allelomorph hypothesis. Therefore they do not call this *tertium quid* a variant of P , but N , and assume that it is independent of P , although they have admittedly never studied the character or its linkage relations.

They do present, however, strong indirect evidence that a third allelomorph of P exists in self races. By crossing two Dutch races (Typical and Deep), each of which in their view carried a different allelomorph of P , and had no other character in common, they expected to synthesize a self rabbit. This they were unable to do, although an F_2 generation of 551 individuals was produced, among which their hypothesis called for 9 selfs. Further, an F_3 generation was produced from selected F_2 animals. Here the expectation for the production of self individuals was considerably higher, but none appeared among 120 young. This result makes it seem improbable that a self rabbit can be produced which contains two doses of either or both allelomorphs of P

which they recognize, for their experiment should have yielded every possible combination of all other associated factors. Self accordingly would seem to require a *different* allelomorph of P than those which characterize Dutch races.

Now if we suppose two Dutch races to contain different allelomorphs of Dutch, and self to contain a third, there is no reason to expect that by crossing the two we should obtain the third (self), any more than by crossing Chinchilla with Polish rabbits, we should expect to get grays, which contain a third allelomorph of the colour factor.

Nor is it possible by crossing White Dutch with self to segregate in F_2 a Dark (Deep) race. One may obtain a *modified* White race, such as the Typical race of Punnett and Pease, and possibly my Tan race, but these are very different from a Dark (Deep) race, as Punnett and Pease correctly observe. Two striking differences are found between the two Dutch allelomorphs. (1) White Dutch and its modifications have much white in the head and frequently have an iris which on its front face is either completely white or white in segments (heterochromia iridis, Punnett). (2) There is a tendency in White Dutch and its modifications to develop numerous fine white spots in the pigmented areas posterior to the belt. Neither of these characteristics is found in Dark (Deep) Dutch, as is agreed both by Punnett and Pease and by myself.

The second conclusion reached in my 1919 and 1920 papers, the existence of modifying factors which make the Dutch pattern darker or lighter than it would otherwise be, has always been entirely acceptable to Punnett. He indeed holds in substance that this is the whole explanation of the variation in the Dutch pattern, aside from the now admitted allelomorphism of P with p . His factors S and T , and the N , X and Y of Punnett and Pease, are nothing but names for supposed modifying factors. No evidence has been presented to show that apart from a Dutch factor proper (P or p) any number of the supposed subsidiary or modifying factors would be able to produce a Dutch pattern.

The extent to which such modifiers may affect the appearance of White Dutch, Dark (Deep) Dutch, and the heterozygote between them is shown in Fig. 2 of Punnett and Pease, p. 378. My own observations are entirely in harmony with theirs as to the large extent of such modifications and their apparent genetic independence of the Dutch factor proper. In my 1919 paper I repeatedly called attention to the fact that parents of high grade regularly produce offspring of higher grade than do lower grade parents of the same genetic constitution as regards the Dutch factor proper.

In consequence of this fact, selection is effective in modifying patterns of the rabbit or of other mammals, as I have long insisted, much to the disgust of supporters of the pure line and pure gamete hypotheses, to whom the idea of "contamination" or "modifiability" is highly distasteful. It is gratifying to receive confirmation in such observations as Punnett and Pease report for their males R 204 and T 35b.

The "Typical Dutch" of Punnett and Pease is by their account only a "modified" White Dutch, since it contains the same Dutch factor proper, p , as characterizes White, and differs only in the modifying factors, S and T . I assent to their explanation except as to the number of supposed modifying factors. I see no reason for assigning the total effect to two factors rather than twelve or twenty. But I do consider it likely that the modifications observed are due to genetic factors independent of the Dutch factor proper, the modifying genes accordingly being located in chromosomes other than that which contains the Angora gene.

Punnett and Pease are in error if they think that their Typical Dutch is the same genetically as the exhibition type of Dutch with which I have worked. The latter corresponds rather with their Mock Dutch, being like it heterozygous for two types of Dutch, White and Dark (Deep).

As to the "Tan Dutch" of my 1919 paper, I am inclined to think, in agreement with the view of Punnett and Pease, that, like their Typical Dutch, it was only a modified White Dutch, containing the same Dutch factor proper and with only modifiers to make its appearance different. For (1) I have found in studies of the last five years that White Dutch extracted from a cross with self may range much lower in grade than it was observed to do in my 1919 studies, extending in fact into the range of Tan Dutch. (2) Even in my 1919 paper it was shown that Tan extracted from a cross with Dark may extend into the lower part of the range of White. (3) After a cross between Dark and Tan, Dark (extracted) was found to be darker and Tan whiter than before the cross. This is in harmony with the view that before the cross Dark contained the *same modifiers* as White, but Tan contained a different series of darkening modifiers. The cross tended to lift some of these darkeners from Tan and add them to Dark. (4) The cross between Tan and White, like the cross between Typical and White of Punnett and Pease, lifted darkeners from Tan and added them to White, bringing the two closer together in grade (mutual modification of my 1919 paper). (5) Tan and White Dutch are similar in appearance, when not very different in grade. Compare Figs. 19 and 21 (Castle, 1919). The head is predominantly white and the iris frequently white. Also, as the belt widens, there is a tendency for the

region behind the belt to be invaded with fine spots of white. (6) Tan originated as a recessive extracted from a self race, whence it emerged in a darkened form, made so by modifiers derived from the self race. White Dutch also is darkened (lowered in grade) by a cross with a self race, as Fig. 2 shows.

In conclusion, I would qualify the multiple allelomorph hypothesis of my 1919 and 1920 papers in only one respect. I am no longer inclined to regard Tan Dutch as containing a fourth Dutch allelomorph; more probably it contained the same allelomorph as White Dutch, associated with modifiers (darkeners) derived from its self ancestors. Unfortunately the race is now extinct so no renewed investigation can be made of its genetic constitution. The other three allelomorphs are in my own stock of rabbits still, and can be obtained quickly by anyone who will take the trouble to cross exhibition Dutch with any true-breeding self race, the exhibition Dutch containing regularly two allelomorphs, and self a third.

THE DUTCH RABBIT—CASTLE, PEASE AND PUNNETT.

By R. C. PUNNETT, F.R.S.

As a test case involving the explanation of a series shewing apparently continuous variation the Dutch rabbit has of late years superseded the much discussed hooded rat. Since it is most desirable that clear ideas should prevail as to the nature and interpretation of this case I need offer no excuse for the following comments upon Professor Castle's discursive criticism of Mr Pease and myself, which appears in the same number of this journal. Fortunately my comments can be brief, because, as will appear below, Professor Castle has provided critical data for a decision between our rival explanations. But before considering these data it may be as well, in view of the continual modifications of Professor Castle's interpretation, if I attempt to outline what at present appears to be its residual form.

We are to suppose the existence of three principal factors **Du**, **du_d** and **du_w** belonging to a series of multiple allelomorphs and corresponding to the pattern types, Self-colour, Dark Dutch and White Dutch respectively. An animal of the constitution **DuDu** is always self-coloured, but Dark Dutch (**du_ddu_d**) and White Dutch (**du_wdu_w**) show a very wide range of variation. The former range from grades 1-7¹, that is from almost self down to the typical Dutch of the fancy; the latter may be anything from grade 2 down to grade 18, that is from a rabbit which is almost self-coloured to one that is almost white. Between the more heavily pigmented White Dutch and the Dark Dutch of similar grades there is, however, a difference. In the former the white "blaze" is usually wider, and they may often show *heterochromia iridis*, which is never the case in Dark Dutch. The cause of this immense range of variation was originally assigned by Professor Castle to what he termed "residual heredity," aided by some mysterious process of "mutual modification." He has now come round to the view that in order to account for this variation we need only invoke modifying factors in the ordinary sense of the geneticist. But he seems to have made no attempt either to analyse or to define these factors. Yet they may bring about

¹ See Pl. I of Castle's *Studies of Heredity in Rabbits, Rats and Mice*, Washington, 1919, which is also reproduced on p. 190 of the present issue of this *Journal*.

an enormous difference in the appearance of a rabbit, for a White Dutch (du_wdu_w) of Professor Castle may be in appearance almost self-coloured (grade 2)¹, or almost white (grade 18) according to its make up in respect of these postulated modifying factors. I may say at once that I have no quarrel with the hypothesis of such modifying factors. Indeed Mr Pease and I may fairly claim to have identified certainly two of them (**S** and **T** of our paper), and probably a third (**N**). Apart from the fact that we have given a reasoned experimental analysis of these factors which Professor Castle is content to leave in a confused jumble, the essential difference between our interpretations is that whereas he invokes a series of three multiple allelomorphs (**Du**, du_d , du_w) to account for what may be termed the major pattern differences, we have found that the facts are covered by the simpler hypothesis of a single factor (**P**). On our view the **PP** animal, in the absence of any of the modifying factors, is what Professor Castle would term Dark Dutch, and the addition of modifying factors brings about an increase in the pigmentation until, in the presence of **N**, **S** and **T**, the animal becomes self-coloured. The self-coloured condition is merely the end term reached when a sufficient number of modifying factors, known from independent analysis, are added to the **PP** animal. On Professor Castle's view the true-breeding self-coloured rabbit is a thing *sui generis*, containing a factor (**Du**) which is not to be found in either Dark Dutch or White Dutch. An animal of the constitution **DuDu** is a self-coloured animal, whatever its condition in respect of modifying factors: an animal heterozygous for **Du** may sometimes be self-coloured, but more frequently shows some white—from which we must infer that every self-coloured animal must have been formed by at least one gamete containing the **Du** factor.

At this point we may turn to an experiment of Professor Castle's to which I particularly drew his attention in 1920, and of which the significance appears even yet to have escaped him. I refer to the cross between Dark Dutch and Tan Dutch (Castle, 1919, pp. 13–15), which on his multiple allelomorph hypothesis is of the nature $\text{du}_d\text{du}_d \times \text{du}_w\text{du}_w$ ². Castle found that the F_1 animals from this cross were on the average darker than either parent, some of them being even self-coloured. A large F_2 generation of 275 animals was bred, showing a range over grades 0–11

¹ Viz. when it is of the form that Castle previously termed "Tan Dutch."

² For Castle has been driven to admit definitely that his "Tan Dutch" is fundamentally a White Dutch with certain modifying factors (cf. p. 195 of the present number of this *Journal*).

on Castle's scale; but the most striking feature was that out of these 275 F_2 animals no less than 89, or nearly 30 per cent., were self-coloured.

This experiment makes it perfectly clear that animals containing only Castle's du_d allelomorph can be self-coloured, provided that they contain the proper independent modifying factors. Moreover if such self-coloured animals were homozygous for du_d as well as for the modifying factors in question, there is every expectation that they would breed true to self-colour. For my own part I feel not the least doubt that in this experiment Castle has achieved what he declares to be impossible¹, and has effected the synthesis of the self-coloured animal from the cross between animals belonging to his Dark and White Dutch classes. As I pointed out years ago this result is just what we should be led to expect on our hypothesis.

In the light of all this, what is the pertinence of Castle's Du factor? Clearly it is an entirely unnecessary assumption unless one is afflicted by the desire to drag in at all costs an interpretation in terms of multiple allelomorphs. The simplest explanation is surely the best. If, as we have already done, Professor Castle will identify his du_d factor with our P , and his du_w with our p , at the same time consigning his Du factor into the limbo of the unwanted, he will then have effected the reconciliation after which he would appear to pine. By doing so, too, he will find the simple way out of the linkage problem that he so triumphantly propounds to us. Self-coloured and Dark Dutch both show linkage with the short hair—Angora pair because both self-coloured and Dark Dutch contain the identical factor P . If S represents the factor for short hair, then it is a case of linkage between P and S , and that is all there is to it.

¹ Cf. p. 194 of the present number of this *Journal*.

SEGREGATION:

BEING THE JOSEPH LEIDY MEMORIAL LECTURE
OF THE UNIVERSITY OF PENNSYLVANIA, 1922.

By W. BATESON, M.A., F.R.S.

(*The John Innes Horticultural Institution.*)

INTRODUCTION.

IN 1922 I had the honour of delivering the Leidy Memorial Lecture at Philadelphia. The substance of that address is embodied in the following paper. I was reluctant to publish it immediately for several reasons. The evidence to which I appealed had not all been fully tested. Some also might be disposed to set aside the phenomena adduced, on the ground that they are exceptional and of small immediate significance pending the determination of the broader principles.

Experience has meanwhile provided confirmation where it was required, and several of the classes of segregation which might formerly be held peculiar or anomalous, have been demonstrated in such abundance that no survey of genetical physiology can disregard them. In so far, moreover, as I was concerned with principle, I wished for opportunities of hearing the criticism of other geneticists holding different opinions; but after many such debates I have learned nothing which appears to dispose of the views to which I inclined.

The growth of genetical science has been surprisingly rapid. To those who have not forgotten the period of stagnation which so long continued, such an activity can only be a source of satisfaction, as implying zeal both in observation and invention. We do well, however, to remember that that long spell of dulness from which we were so lately emancipated, ensued as the direct consequence of a too facile acquiescence in impermanent doctrines. Curiosity was too easily allayed. We are in no such danger yet, but the following pages may at least serve as a reminder that, even as regards the outline of genetical principles, finality has not been attained.

1. THE NATURE OF SEGREGATION.

Segregation is the central phenomenon round which the modern conceptions of genetics have been developed. Two questions regarding segregation may be asked with some hope that observation may provide an

answer. First, what in the act of segregation separates from what? Secondly, when, at what moment or moments in the life-history of an animal or plant, does this separation occur?

In the first of these questions we are obviously considering a matter of fundamental importance. Let us begin with segregation in respect of any factor in the homozygous state. Whichever the critical division may be, it normally then results in the formation of two similar products. All that we know is consistent with the belief that segregation in this case is the segregation of two *similar* things, upon whatever their ultimate properties depend. Here the conception of allelomorphism raises no difficulty. Whatever is taken by the one half resulting from the critical division, is identical with that which is taken by the other half. But when segregation is effected between the dividing cells of the heterozygote, the consequence of that process is that the two halves differ in some demonstrable respect, being unlike in their genetical powers. Though wholly ignorant even of the proximate elements upon which these powers depend, we are nevertheless tempted to form some conception of the nature of the unlikeness between the resulting halves.

We distinguish at once two possibilities. The distinction between the two halves may be due to the presence of something in the one which is absent from the other, or it may be attributed to the presence of a different positive and effective determinant in each of the two halves. The first of these interpretations is known briefly as the Presence-and-Absence hypothesis. From an early stage in the development of Mendelian analysis that supposition has seemed to me a reasonable one, furnishing, as it does, a concrete and acceptable account of the nature of allelomorphism—a relationship otherwise mysterious and paradoxical—with the additional merit that the basis for a simple and widely applicable notation is immediately provided. This representation has the advantage of extreme simplicity. It implies only that something, whether a material something or otherwise, is present in the one half which is absent from the other, and it is equally applicable whatever the future may reveal as to the ultimate nature of the factors, and whether the distinction prove to be one of material, or, for example, to take another possibility, of potential.

With the supervention of the chromosome theories, for reasons which I have never clearly understood, the interpretation based on Presence-and-Absence was set aside. Objection can scarcely be taken on the ground that no cytological distinction is normally visible between the two halves. Though intelligible as a reminiscence of the period in which

whole chromosomes were looked upon as possibly responsible for separate factors, that consideration has little weight now that every chromosome is regarded as the possible bearer of countless genes. The expectation that the absence of even a large number of genes might make a difference distinguishable to the eye, with the means at our disposal, is one that even the crudest form of the hypothesis of transmission by chromatin would scarcely lead anyone to entertain. Moreover, whatever cogency such argument might have possessed so long as the male *Drosophila* was believed to have no Y chromosome, disappeared when a Y was found and, though present, was found to be devoid of genes.

The contrast between the two modes of representation reaches a climax in the expressions which we should respectively use to denote the genetical constitution of two white-flowered races which, as in Sweet Peas, may when crossed give a coloured F_1 , followed by 9 coloured : 7 white in F_2 . For Morgan the two whites, though alike and indeed indistinguishable without the breeding test, "are the product of different factors¹." To complete the statement I suppose we might next declare that the white in F_2 which gives no colour when crossed with either parent race is the product of the two different factors for white combined. The same reasoning would be applied to all examples in which two similar recessives when crossed reproduce the normal dominant. Surely the invocation of these positive elements to "produce" the recessive character is here evidently superfluous. The whites are all alike whites. Some are reduced to whiteness through the loss of one of the complementary elements—in our notation *C*, some inasmuch as they are destitute of the other, *R*, others again inasmuch as they have lost both. Nor is the distinction one of words merely, for according as one or the other representation is adopted our conceptions of genetical physiology will be greatly changed.

The argument most frequently specified as militating against Presence-and-Absence is derived from the phenomenon of multiple allelomorphism. When Johannsen², for example, adopts the American view, speaking of the Presence-and-Absence hypothesis as "now abandoned," it is upon the multiple series that he apparently bases his judgment. Rightly regarded, however, I contend that the multiple series not merely contribute nothing capable of such construction, but that their existence amounts almost to a demonstration of the correctness of the Presence-and-Absence hypothesis.

¹ *Mechanism of Mendelian Heredity*, ed. 2 [1923], p. 36.

² *Hereditas*, iv. p. 138, 1923.

In developing his criticism of that hypothesis, Morgan remarks that "it is a characteristic of 'multiple allelomorphs' that the same character is affected¹." Apart from exceptions not yet well defined, this is true, and the fact is of great significance. The statement should be amplified, and may be put in the form that, apart from the exceptions, factors composing a multiple allelomorphic series produce an effect in degrees quantitatively different. The factors composing any such series may thus be arranged in a descending scale. This is exactly what is expected. The factor put in on the one side is absent, wholly or in part, from the other, and, on segregation, the two qualities which respectively were combined in fertilisation, reappear. Why Morgan should declare that "only one kind of absence is thinkable²," I do not understand. We should not assert that because a sovereign is absent from a purse, that purse must contain nothing. Surely my opponents must sometimes wonder why, according to the views they entertain, the relation of allelomorphism so constantly exists between positive and negative in a single respect; since, if they deny these relations to be a matter of quantity, allelomorphism might be expected to manifest itself just as often in a relationship between characteristics having no perceptible connection with each other.

When we find, as we do in a series of Multiple Allelomorphs, that the relationship holds, not only between one pair of quantitative differences, but between a whole graduated series of such differences, the nature of that relationship might be regarded as settled beyond dispute. We have been asked to conceive of each allelomorph in any pair as equally a positive something. In a multiple series, are we then to suppose that as the allelomorph of *each grade* in the series a corresponding positive something exists? Such an interpretation verges on absurdity, and the multiple series are a clear demonstration that allelomorphism is not a haphazard relationship between two independent features, and as good a proof as we can possibly expect, that that relationship involves and implies a distinction in some quantitative respect.

The quantitative distinction may of course manifest itself in various ways. The consequence of fractionation may appear, for example, as either a diminution of the area affected, or in a reduction of intensity of an effect, and doubtless in other ways also.

When the nature of these quantitative series is properly understood we obtain a simpler and clearer conception of the meaning of factors for

¹ *Mechanism of Mendelian Heredity*, ed. 1915, p. 168; ed. 1923, p. 215.

² *Physical Basis of Heredity*, n.d., p. 251.

pattern, for extension, for intensification or dilution, and the like, which now form so large a part of our analytical apparatus.

As the simplest possible illustration of how the quantitative hypothesis may be applied to the representation of a multiple series, take the yellow "eye" of *Primula sinensis*¹. As a matter of history the middle term in the series is the original normal. The eye extends over about $\frac{1}{4}$ or less of the limb. In a recessive form, "Primrose Queen," it extends over about $\frac{1}{2}$ of the limb. The form with more white is completely dominant. Obviously we may say that there is something present in this organism which represses the development of the yellow in the middle third. Later appeared "Queen Alexandra," in which there is barely a trace of the yellow eye. Whichever of the first two is crossed with this top dominant, the result is the same, and the eye of F_1 is almost or quite as pale as the eye of "Queen Alexandra." The three terms behave as multiple allelomorphs:

W makes the whole petal white, as in Queen Alexandra.

$W - \frac{1}{4}$ (as an approximation) leaves the band of yellow in the normal.

$W - \frac{1}{2}$ allows the extension of the yellow over a further segment, as in Primrose Queen.

Possibly W may be still further diminished, but representation of the terms already known suffices for present purposes. Subsequent discovery of other terms in the series can in no way disturb the applicability of this system. So long as nothing lower than Primrose Queen is known, that may be taken as the basal negative.

Colour in the Rabbit may be taken as an illustration of somewhat greater complexity. Self-colour with dark eyes, the Himalayan and the albino, both pink-eyed, together form a multiple series. From whatever parentage the albino be derived, it cannot, crossed with the Himalayan, produce a dark-eyed or a self-coloured form. On the other hand, the allelomorphism of self, Dutch, both dark-eyed, and the albino does not involve a multiple series. Dutch crossed with albino gives either Dutch again, or an approximately self-coloured animal, according to the factorial composition of the albino. The nature of this distinction may be very simply expressed. We may describe the Dutch and the Himalayan as each wanting in some portion of the full complement needed to confer self-colour, but the ingredient missing from the Dutch is not the same as that missing from the Himalayan. Nor are the factors for these two ingredients allelomorphic to each other. If we call C colour and S the

¹ See *Journal of Genetics*, XIII. p. 226, and Pl. XVII, fig. 35, 1923.

element conferring self-distribution, then the Dutch has $C\frac{S}{2}$ and the albino either cS or $c\frac{S}{2}$,—the one capable of turning Dutch into Self, the other leaving it Dutch¹.

But the Himalayan is deficient in another ingredient. For simplicity we suppose this to be the C spoken of above. The $C, \frac{C}{2}, c$, form a multiple series; and the Himalayan bred to the Dutch has the power of making F_1 Self, since, though having only half of C , it has the whole of S . Thus:

<i>Dutch</i> × <i>Himalayan</i>				
		$C\frac{S}{2}$	$\frac{C}{2}S$	
	F_1 Self	$C\frac{C}{2}$	$S\frac{S}{2}$	
F_2	<i>Self</i>	<i>Dutch</i>	<i>Himalayan</i>	<i>Dutch Himalayan</i>
	cS	$C\frac{S}{2}$	$\frac{C}{2}S$	$\frac{C}{2}\frac{S}{2}$

In F_2 there are of course animals, otherwise Himalayan, but deficient in some of the colour proper to a correct Himalayan. They are mentioned by Punnett² as "full Dutch Himalayan" and they have more or less white on those parts which, though coloured in correct Himalayans, are white in the Dutch. Evidently all albinos contain S in some degree, though the quantity may be small and result in the very low terms in the Dutch series like those which Castle has figured³. If albinos altogether devoid of S existed, we should have 9 coloured : 7 white in F_2 , and, subsequently, extracted albinos capable of giving coloured offspring in their crosses, neither of which results has been seen in rabbits. The fact that an albino may contribute a factor which can raise the Dutch up to self-colour proves that after all the colour of the animal is like that of the plant, in that it is due to the interaction of complementary elements, although since one of them is never wholly absent, we do not meet with the production of colour from two albinos. Self and the

¹ The expression $\frac{S}{2}$ is not intended to mean that the factor S is exactly halved in the Dutch. As in some similar examples, the fractionation of this factor is very indefinite. Probably no strain, even of thoroughbred Dutch, shows uniformity in correct marking; and, as Castle has shown, there are very many grades which have overlapping fluctuations. The Primula eyes are indeed somewhat exceptional in the sharpness with which their quantitative grades are defined.

² *Journal of Genetics*, II. pp. 237-8 and Pl. XIV, fig. 2, 1912.

³ *Carnegie Inst. Publ.* No. 288, Pl. I, 1919.

various grades of Dutch are probably related as a multiple allelomorphic series, though, as explained above, the graded factor is not that which is responsible for the self-Himalayan-albino series. When the chemistry of colour-production is understood more fully, we may find that the element in the animal which I here speak of as *S* may be analogous with one of the two complementaries of the plant; and that the distinction between the self and the picotee in the Sweet Pea is due to quantitative differences in an element playing the same part as that which by its quantitative grades distinguishes the self from the Dutch in the rabbit.

When we come to the mouse, a further term appears. For the pink (really *pale*) eyed mouse, with dilute-coloured coat, crossed with certain albinos, may produce dark-eyed F_1 (whether self-coloured or piebald). The allelomorphism is not here multiple, and we are thus precluded from regarding the pale eye and the weakness of the coat colour as simply degradations of the rabbit's factor *C*. Nor are they due to one of the stages of *S*, for that is concerned with the area of the coat-colour. For clearness we must use a fresh term, *D*, the factor which can turn the pink eye dark, and the coat deeply coloured, when it is in combination with *C*. So the pale-eyed with light coats are *pC*, and the albino is either *pc* or *Pc*.

We cannot as yet positively declare how these factors in the Mouse are related to those in the Rabbit. Sewall Wright¹ has done this with considerable success for most of the series in mammals, but I do not understand that the particular problem here in question is dealt with in his scheme.

When the factorial scheme is thus set out, we obtain a clear suggestion of the nature of a "pattern factor." The colour of the Dutch Rabbit has its peculiar distribution because some ingredient necessary to the formation of pigment is actually missing from the parts which remain white. One of the ingredients—say the enzyme—is not broken up piebald-wise, but extends over the whole skin and eyes. The other, presumably the chromogen, is wanting from the white areas. Rabbits do not, I believe, show ghost marks, but where those marks exist I can scarcely doubt that they indicate the actual presence of a material due to the activities of some factor, which comes thus to be regarded as responsible for a pattern. The question, which ingredients can be broken

¹ *Amer. Journ. of Hered.* viii. pp. 224, 373, 473, etc. The graphic method of representation which he has suggested has great advantages, and comes nearer to giving a rational and acceptable picture of the phenomena than any I have seen elsewhere. (See especially *l.c.* p. 230.)

up and which cannot, has probably much bio-chemical significance. We may declare, for example, that the existence of tricolour guinea-pigs means that in that animal not only the chromogen but also the element which can turn red into black, may be broken up into piebald areas, whereas the non-existence of tricolour mice and rats means that in them this element (Sewall Wright's Enzyme II), or its product, always pervades the whole skin. Similar interpretations are probably applicable to all pattern factors, and bring them at once into accord with the quantitative system of representation.

The quantitative system commonly obviates the hypothesis of complete linkage, to which recourse is sometimes had by those reluctant to recognise the existence of multiple series.

This appeal to linkage, as it seems to me, leads to further embarrassments. If a multiple series really relates to "the same character," the quantitative system is a sufficient, and, as I think, a more probable representation of the phenomenon. I know as yet of no series proved to be multiple, in which the several terms in the factorial series have effects manifested in apparently unrelated physiological consequences. The series most nearly fulfilling this condition is that originally discovered by Nabours in *Paratettix*. The distinctions there, as Morgan says, are all matters of "colour"; but when colours not merely take the form of reds, yellows, etc., which can reasonably be interpreted as modifications of a single pigment, but include also the development of a dense white, obviously consisting of a wholly distinct material, that coloration is not readily to be accepted as a single character. That in Nabours's segregation linked factors, or groups of factors, are concerned (in addition to multiple series), seems highly probable; and indeed, in the later phases of his work, some distinct evidence of crossing-over was obtained. Pending an extended study of the genetics of *Paratettix* the decision must remain in doubt, and I am willing to admit that there may here be something exceptional.

Apart from exceptions probably capable of special explanation, the quantitative mode of expression clearly and sufficiently represents a very wide range of genetic phenomena. No hypothesis whatever is as yet offered as to the nature of the factors, as to the manner by which they produce their effects, or as to the regulatory process by which, in such manifestations as those of colour-pattern, the effect is directed with more or less constancy into special regions. For this last phenomenon I have no doubt a simple mechanical cause will hereafter be discovered, and it will then seem no more remarkable that the pigment of the Dutch

or of the Himalayan Rabbit should occupy their respective positions, than that the shapes assumed by floating drops of oil should be influenced by the constitution of the fluid on which they rest, or their positions of rest by the shape of the vessel.

Johannsen has recently discussed again the conception of units and taken views somewhat different from those to which I have inclined. In so far as his criticisms lead to negative conclusions, the difference between us is, I suspect, verbal only. The term "unit-character," to which Morgan also takes prominent exception, means of course a character brought into being by the operation of an element or factor which segregates as a unit. No confusion is introduced by the abbreviation. By associating that term with the notion of "unit-parts" Johannsen implies that our present ideas are merely an amendment of those involved in the old debate on "prae-formation or Epigenesis," whereas modern genetics has the special merit of obviating the need for such discussions. He adds: "Nowadays each of Bateson's allelomorphs are [is] not regarded as a kind of germ ('*Anlage*') for a corresponding 'unit-character'." I cannot imagine how anyone should at any time have so regarded them. For me they have always been simply ingredients, contributions to the collective stock which makes up the organism. Nor can I agree with Johannsen when he says: "The worst of all these relics is probably the expression *Transmission*, where no transmission exists but where continuity is found." If we had to re-make our language, we might with slightly greater precision say that a unit was *de-mitted*, but to say that it is transmitted or sent through to the offspring introduces no wrong metaphor, and accords well with the quantitative representation of units. One of the most valuable principles to which Mendelism introduces us is the fact that an organism can only pass on what it itself possesses.

In one of their contentions my critics are right. The course of the evidence may convince us that of each allelomorphic pair one is positive and the other negative, but we have not yet the means of distinguishing with complete certainty which is the positive and which the negative. Ten years ago I called attention to this paradox¹. With sufficient perversity the albino may be symbolically represented as the positive, and the normal as the negative. If *R* is a factor which when homozygous inhibits colour, then the albino is *RR* and the coloured forms are either *Rr* or *rr*. If dominance is complete I suppose few would favour this mode of representation, since we all but know that it is nonsense; but when

¹ *Pres. Address to Brit. Ass.*, Melbourne, pp. 17-18, 1914.

dominance is, as often, imperfect, I grant that we have no satisfactory criterion for distinguishing positive from negative and can only guess from analogies. I may guess that the white element in the Blue Andalusian is a peculiar dominant white, partially inhibiting the black, but I am not at all sure it is not a peculiar recessive white, diluting the black: and we have all seen plenty of examples, where such characters as leaf-shape are involved, in which we cannot yet hazard a surmise, or attempt to allot the positive and the negative parts. But the appeal to this present imperfection of knowledge does not touch the essence of the problem, and the Presence-and-Absence or quantitative conception is in such complete harmony with the tenour of the evidence, that I have no serious doubts of its correctness.

Mohr and Wriedt¹ have raised a question of interest at this point. Discussing a peculiar brachydactyly they suggest that the organism homozygous for the abnormality may be non-viable, and incline to regard one of their records as exemplifying that condition. Now if one dose of normality were needed to keep the organism alive we might, without straining the evidence, regard it as an inhibitor, the *absence* of which had, in double dose, a lethal effect. Surprise has often been expressed that human abnormalities should in such a large number of instances descend as dominants. Nothing comparable is known for any other organism. To interpret all these as examples of the addition of new positive elements is difficult. On the lines of the new suggestion a not unacceptable alternative is provided.

The *Drosophila* workers having rejected the whole scheme on general grounds which, as I have argued, seem insufficient, claim further that some of their special experiences are not reconcilable with the Presence-and-Absence hypothesis. Without intimate acquaintance with the phenomena I cannot form an independent opinion as to the validity of these objections. Apart however from them, in a wide survey of the genetics of plants and animals I find nothing very seriously conflicting with the quantitative representation of Mendelian distinctions.

In the very remarkable paper of Bridges² describing triploid intersexes in *Drosophila*, a new and attractive conception is introduced which should lead to further developments. According to this system, sex is determined by a *balance* between the powers of the X-chromosome and

¹ *Carn. Inst. Pub.* 1919, No. 295.

² "The Origin of Variations in Sexual and Sex-linked Characters," *Amer. Nat.* LVI. p. 51, 1922; and *Sci. N.S.*, LIV. Sept. 16, 1921. A fuller development of this conception of "balance" will be found in the new paper by Bridges, *Amer. Nat.* LIX. p. 127, 1925.

those of the autosomes, especially that of the IVth chromosome. A graduated series is thus constructed, ranging from a form with 3 X -chromosomes and 2 sets of autosomes—which is called a “super-female”—down to a “super-male” with a single X -chromosome and 3 sets of autosomes. If I might call the bottom term an *infra*-male, I should regard the series as an excellent example of the quantitative system of representation.

The existence of such a series renews the hope that a way may yet be found of bringing sex-determination by a “heterogametic” male (*Drosophila*, man, etc.) into harmony with that by “heterogametic” females (moths, birds). Since in *Drosophila*, etc., female is XX and male X , if in the moth female were X and male zero, the whole would form one quantitative series. From this simple account we are barred, not merely by the observations of Seiler that the male moth has two visible X 's, but by the universal experience that in moths and birds the male may be homozygous in sex-linked factors, and thus, by inference, in X -chromosomes. I know no other so serious objection to the quantitative hypothesis. The two types of sex-determination, however, constitute an outstanding puzzle, with which no theory of evolution has yet satisfactorily coped.

2. THE MOMENT OF SEGREGATION. ANISOGENY.

The work of the Columbia school has shown beyond possibility of doubt that in animals the reduction division must be the moment at which segregation in respect of Mendelian factors is usually effected. These—the transferable factors—must be in some way associated with the chromosomes. We have no idea what the nature of that association may be, but that it is a reality the discoveries built on the facts of non-disjunction sufficiently prove. Especially convincing are the preparations proving that certain features in *Drosophila* appear exclusively in those insects which have the IVth chromosome haploid, and that other distinctions occur regularly where the same chromosome is triploid. The demonstrations of those facts which Dr Bridges was good enough to give me in December, 1921, would dispose of the most inveterate scepticism.

But if we press for a more exact account of the nature of the association subsisting between factor and chromosome, no answer is forthcoming. That the particles of which the chromosomes consist should by virtue of various chemical composition possess these highly varied factorial powers, appears in an extreme degree improbable. As to the mechanism by which crossing-over might be effected, we have scarcely

any plausible suggestion, and none which is in certain accord with cytological observation. Further discussion at the present stage is unfruitful. We await a fresh light.

The fact that the chromosomes are observed in the cell-divisions of tissue-cultures to disappear¹ altogether after telophase and to pass into solution, makes the application of the conventional idea increasingly difficult, though providing unlimited possibilities in fresh directions. If the genes are, or are attached to, particles of material grouped in a more or less permanent arrangement in the chromosomes, and if these particles return to their serial places after dissolution of the chromosomes, in preparation for the next mitosis, we may have to do with a behaviour of matter hitherto, I believe, unrecognised by physicists.

Apart from difficulties of a fundamental character, we meet with various indications which, though capable of a logical and formal reconciliation, with the simple chromosome theory, are not quite readily explained away. As measured by the frequency of cross-overs the assignment of factors to definite positions is open to errors of many kinds and unfortunately at present we have no other method of investigating this problem.

As various evidence has shown (especially that of Mavor², working with X-rays), the number of crossovers may be influenced from without, and whether the change is due to alteration in the position of the gene, or to disturbance of the mechanism of crossing-over, cannot be clearly distinguished. The experience of all geneticists is that some crossover values are liable to great fluctuation, and the linkage of one factor with another may even prove to be a transient association, as Hammarlund saw in *Pisum*. He fertilised a double recessive (white flowers, yellow pods) with pollen from a double dominant (purple flowers, green pods) and raised 10 F_1 plants whose offspring gave a normal 9 : 3 : 3 : 1, with no linkage. Using as polleniser a double dominant, sister to that used in the first series, he raised 13 F_1 plants which showed a clear 63 : 1 linkage; and with the same pair of parents crossed reciprocally he raised 19 more F_1 which showed the same linkage³. I suppose these facts must be taken to show that one or other of the two genes which were in the same chromosome in one plant, were in different chromosomes in its sister. No one is in a position to deny the possibility, but it opens up a bewildering prospect.

¹ Strangeways, T. S. P., *Tissue Culture in Relation to Growth and Differentiation*, Cambridge, 1924.

² Mavor, J. W., *Proc. Soc. Exp. Biol. and Med.* xx. p. 335, 1923.

³ *Hereditas*, iv. p. 235, 1923.

In our work on the linkage of *Primula sinensis*, another unaccountable fact has appeared. In this hermaphrodite plant, the values for certain linkages differ considerably on the male and female sides. The extreme cases are as follows:

	Observed linkage		Percentage of Crossovers	
	Female	Male	Female	Male
Short style and blue in flower	12.2 : 1	7 : 1	7.5	12.5
Green stigma, light leaf ...	29.6 : 1	52.4 : 1	3.2	1.8

Any attempt to account for the pairing of these allelomorphous elements in the conjugation of the parental chromosomes encounters serious difficulties—the more so since the closer linkage is in the first instance on the female side, and in the second on the male. All four factors are in the same linkage-series.

The *Drosophila* workers alone can decide whether in the course of their experience analogous complications have arisen, but provision for much elasticity and emendation is needed to bring the chromosome theory into accord with the facts of plant genetics.

On a wider survey also of the facts of heredity we see indications that the conception of linkage provided by the chromosome theory, though probably containing an essential truth, is in some important respect imperfect. We have, for example, to recognise that in segregation blocks of characters may exhibit a coherence not readily attributable to that random collocation of genes in single chromosomes, which is the only type of linkage contemplated by the theory. An exceptionally striking instance of this phenomenon was seen by Engledow in families derived from the cross Polish wheat \times Kubanka¹. He enumerates ten characters of the grains and glumes which thus cohere and are as he terms them “inseparables.” They may no doubt, as he remarks, be all controlled by one factor, and for some, as for instance the lengths of grain and glume, this may easily be supposed. Nevertheless others, such as the ribbing of the glume, its consistency, and the numbers and length of the hairs at its apex, would naturally be regarded as independent. It may be suggested with plausibility that this segregation in blocks is to be attributed to the fact that the cross in question was made between parents which have some claims to be regarded as real species, and that the association of characters may be due to an association of particular chromosomes. The evidence should be watched for signs of any such behaviour as a characteristic of species crosses. If established as of at

¹ *Journal of Genetics*, x. p. 124, 1920.

all frequent occurrence, the generalisation might be of much significance¹. Meanwhile, so far as *numbers* of chromosomes are concerned, it may be remarked that Kubanka and Polish are alike, with $n = 14^2$.

When a genetic analysis of human characteristics becomes possible I have no doubt that many such examples will appear. What we know of the transmission of family likenesses both in physical and mental attributes is not easily consistent with the theory of random assortment in chromosome groups. In controlling the form of the orbit, of the alae, and of the lips, features upon which human resemblance especially depends, we cannot doubt that countless genetic elements take part. That this is true is proved by the easily observed fact of recombination, and yet strong likenesses extending to features of all kinds shared by many members of the same family groups are to be observed every day. This is scarcely consistent with the supposition that the elements co-operating to produce these miscellaneous effects are wholly independent in their transmission; but we have equal difficulty in interpreting these extensive associations as merely manifestations of chromosome linkage.

If referred to chromosomes, these associations of factors suggest rather a partial grouping of particular chromosomes than a collocation of factors within individual chromosomes, but the consequences of the two processes may readily be confused.

Goodspeed and Clausen's *Nicotiana* crosses are very suggestive of such association. Reciprocal crosses between six different varieties of *N. tabacum* and *N. sylvestris* gave F_1 resembling the particular *tabacum* variety used. These F_1 plants had sterile pollen, but the female side could be back-crossed with the parental species. With *sylvestris* father they gave two classes of offspring: (1) aberrant plants which were almost sterile and gave no seed when selfed; and (2) plants partially fertile closely approaching *sylvestris*, which on selfing in successive generations soon produced a fully fertile line not distinguishable from *sylvestris*³.

The converse combination, $F_1 \times \textit{tabacum}$, gave three classes: (1) aber-

¹ W. H. Gates has lately published a preliminary account of crosses between Japanese waltzing mice and house mice in which the characters similarly segregate often in blocks. Evidence is added indicating that the Japanese are *Mus wagneri*, regarded as a distinct species. *Proc. Nat. Ac. Sci.*, vol. xi. p. 165, 1925.

² In plant-sports somatic segregation by blocks is occasionally seen. Sageret (*Ann. de la Soc. d'Hort. de Paris*, II. Livre 7, p. 159, 1828) describes in detail such an example in a melon which bore two fruits differing from each other in several respects of structure, colour and flavour.

³ *Amer. Nat.* LI. p. 31, 1917.

rant nearly sterile forms producing no seed when selfed; (2) forms like *tabacum*, also almost sterile; (3) plants partially fertile, resembling *tabacum*, which on selfing resulted in a fertile strain like the original *tabacum* in appearance. In a recent paper Goodspeed gives an account of the cytology of these hybrids. *Sylvestris* has $n = 12$ and *tabacum* $n = 24$. In F_1 therefore twelve chromosomes go unpaired. Here therefore the fact that the fertile derivatives are those which most closely reproduce the parental types is interpreted by the observers as meaning that only a few of the chromosome combinations are sufficiently harmonious to be effective, and that these are approximately those which constituted the original species. The comparative fertility of the female side of F_1 is the chief obstacle to this interpretation, but if not unduly pressed, the scheme may be accepted as being in general accord with probability.

Somatic Segregation.

Though we can no longer doubt that segregation is, perhaps by more than one process, commonly effected at the reduction division, evidence steadily accumulates showing that at least in plants of many kinds comparable segregations occur in somatic divisions also. When we first knew that linkage was an expression of parental association of characters, Punnett and I suggested that by some process of vegetative reduplication, the cells bearing the parental as opposed to the crossover combinations became more numerous. The chromosome theory obviously provided an alternative account which we admit to be so much more probable that the conception of reduplication fades inevitably into the background. Whether reduplication ever happens at all as originally suggested is doubtful. Such a consequence might not improbably ensue in the growth and multiplication of material like the germinal tissues, *commonly devoid of axes of symmetry*, but admittedly we cannot point to any observation which is conclusive. I notice, however, that both Nilsson-Ehle in Wheats, and Heribert Nilsson in *Oenothera* have met with phenomena which they incline to regard as examples of reduplication.

Anisogony.

Breeding tests have proved that in heterozygous plants of many kinds the pollen and ovules are unlike in the genetical contributions which they make to the composition of the next generation. The first example was discovered in Miss Saunders's experiments on single "double-throwing" Stock (*Matthiola*), the pollen of which bears exclu-

sively the recessive doubleness, whereas the ovules are mixed, some bearing singleness, others the recessive. The same rule was soon ascertained to hold good of the white and cream plastids in the white race which continually throws creams, and again the pollen bore exclusively the recessive. This phenomenon is so common and is the subject of such constant reference that a special designation for it is required. We have sometimes spoken of such distributions as "unilateral," as opposed to the ordinary or "ambilateral" distribution in which both sides, male and female, equally participate. These terms are open to certain objections, especially on the ground that inasmuch as the female side generally, perhaps always, carries a mixture, the distribution is not strictly one-sided. The choice of a term is rendered difficult from the absence of agreement as to the physiological nature of the distinction between the two sides. I should wish to express the fact that there has been a *separation*, such that the one allelomorph passes to one side wholly or predominantly. Those, however, who regard all genetic segregation as effected at reduction, have put forward alternative interpretations. For them the appearance of separation at some somatic division is spurious; and the absence, for instance, of singleness in the pollen of *Begonia Davisii*, is taken to show not that the singleness is not represented among the grains, but that the grains bearing it are by some unknown process rendered inoperative. Some writers use the expression "heterogametic" in application to these plants; but the term (as well as "heterogamic") is already in general use both in zoology and botany, with several distinct meanings, of which perhaps the most usual and important is in application to the heterozygous sex of animals—the male or the female, as the case may be. For this use, in which it is entirely appropriate, the term "heterogametic" might be maintained exclusively. Pending clear evidence as to the nature of the phenomenon, it may perhaps be best described as *anisogeny*, a term which merely declares that the contributions of the two sexes are unequal, in contrast with the normal or *isogenous* condition in which they are alike.

Besides those in the Stock, already mentioned, the most familiar examples are those gyno-dioecious plants in which the females fertilised by pollen of hermaphrodites give entirely or predominantly females. This was, I believe, first recognised by Correns, though no interpretation was at that time offered. In collaboration with Miss A. E. Gairdner, I have investigated a simple and particularly instructive example of anisogeny in *Flax*. Ordinary flaxes, both fibre and oil varieties, are regularly hermaphrodite. A peculiar dwarf form which appeared here

spontaneously¹, is similarly hermaphrodite, but fertilised by any fibre flax it gives in F_2 an ordinary recessive, characterised by male sterility, complete or less often partial. Fertilised by fibre flaxes, these recessives give nothing but similar male steriles, whereas, fertilised by pollen of the dwarf, they give all hermaphrodites in F_1 , which in F_2 give a normal 3 hermaphrodites : 1 sterile.

The obvious and most simple interpretation of these facts is that in the fibre flaxes the element which determines hermaphroditism passes into the female side of the plant, leaving the pollen devoid of this factor.

Analogous evidence has accumulated from various gyno-dioecious plants, but flax is peculiar in that, although the heterozygous combination in the normal flax segregates upon the anisogenous plan, nevertheless if the hermaphrodite factor be introduced from the dwarf variety, the heterozygote is isogenous and a normal 3 : 1 results. I can suggest no reason for this distinction.

In *Campanula carpatica* Miss Pellew has found that anisogeny governs not only the anther-development, but also, less completely, the factor for blue colour as opposed to white.

In none of these examples do I see any difficulty in attributing the anisogeny to some somatic segregation occurring in the course of the development of the flower.

The advocates of gametogenesis as the exclusive seat of segregation have made several alternative suggestions, invoking lethal factors acting either on gametes or on special zygotic combinations, differential properties of gametes of special constitution affecting their germinative or fertilising powers, and similar discriminating influences; but so far I am not aware of any positive or material evidence pointing in these directions. Admittedly no decision can be made with complete certainty as yet, and in the next examples complications exist which have not yet been elucidated. In *Begonia*, as in *Matthiola*, anisogeny in respect of double flowers² is met with. Most remarkable in this respect is the case of *Begonia Davisii*, a wild species which, though breeding true to singleness,

¹ We have never succeeded in tracing this variety. It is a *usitatissimum* and perhaps is somewhere cultivated for oil (see *Jour. Gen.* xi. p. 269, 1921).

² *Petunia* has been sometimes quoted in this connection, but, as now appears, in error. Doubles are functionally male only, having no ovaries. They are always raised by fertilising singles with the pollen of doubles. A mixture of singles and doubles results, of which the singles breed true. Miss Saunders, on the analogy of Stocks, took the doubles to be recessive, but, as Frl. von Ubisch pointed out, the double may more simply be taken to be a heterozygous *dominant*, like the ordinary double *Carnation*, on which interpretation no problem arises (*Zeits. f. Botanik*, xv.).

gives exclusively double flowers when its pollen is used to fertilise the female flowers of doubles¹. Definite anisogeny has been found to exist in *Begonia semperflorens*², affecting the distinction between red and pink flowers. Pink is dominant, and commonly the male side of the heterozygote gives 1 pink : 2 red, whereas the female side gives equality. Both these proportions have been determined by back-crossing on a large scale, but for some reason unknown, the heterozygotes *selfed* have given approximate equality (2782 pink, 2536 red; or 1.1 : 1) instead of the arithmetical expectation 2 pink : 1 red. Such a fact may be construed as an indication of selective action. In connection with it should be mentioned the production of a small but significant proportion of slightly petalodic plants in the cross *Begonia Davisii* ♀ × double ♂. Since *B. Davisii*, itself fertilised by its own pollen, gives no double flowers, and, as we have just seen, its pollen judged by back-crosses must be supposed to bear doubleness exclusively, the origin of these petalodics is problematical and may conceivably indicate that the eggs producing them do not, for some reason, take part in the normal descent of the species by selfing or *inter se* fertilisation³. What the meaning of these unconformable results may be, we cannot say, but they must be mentioned as in some degree throwing doubt on the simple interpretation of anisogeny to which I incline.

A feature which, as cases multiply, assumes a certain significance is the fact that in all the examples of simple anisogeny yet discovered in plants the peculiar property of the pollen is to carry the recessive. *Petunia* used to be quoted as an instance to the contrary, but as remarked, that interpretation has now lapsed (p. 217, note). Attempts to build up an anisogenous plant in which the properties of the male and female sides should be reversed have not, so far as I know, been as yet successful.

Among the *Oenotheras*, as de Vries first showed, anisogeny prevails extensively. It is there combined with the further complication that the characters may be associated in groups or blocks which are difficult

¹ W. Bateson and Ida Sutton, *Journal of Genetics*, viii. p. 199, 1919. During the past six years these experiments have been greatly extended by Miss de Winton. Not merely has the original *Davisii* this anisogenous distribution, but singles derived from its female side successively fertilised by doubles continue to behave in the same way for several generations unchanged.

² This is a subject on which I have been engaged for several years in collaboration with Miss D. de Winton.

³ We cannot easily attribute the petalodics to some more extreme doubleness brought in by the double used as male, for *Davisii* itself, used as a male, is evidently genetically a thorough double. Nevertheless that possible interpretation must be remembered.

to interpret as examples of ordinary linkage. The further investigation of Renner and other *Oenothera* specialists have greatly increased our knowledge of this subject, but the facts are so complex that a general idea of the genetics of these plants is all that those unfamiliar with the material can form. In the abundance of dead pollen grains so commonly seen in them we have here palpable evidence of special complications, added to which, lethal zygotic combinations almost certainly play a considerable part. For these reasons we can scarcely appeal to the *Oenotheras* as elucidating the anisogeny of plants in which none of these disturbing elements have as yet been demonstrated.

Among the phenomena which Miss Irma Andersson¹ has observed in studying the genetics of variegated ferns are several which illustrate a segregation among somatic cells in respect of the condition of the chloroplasts. In one example especially the behaviour offers a remarkable analogy to what is here called anisogeny. Variegated *Scolopendrium*, characterised by bands of pale yellowish green, produces from its spores prothallia either fully green or in various shades of paler green. The fully green prothallia stay green and give rise to ferns which are wholly green and breed true. The pale prothallia rarely range up to green, and when bred together give variegated ferns only, no greens and no whites. But the most curious feature in this case is that the 64 spores in any one sporangium are commonly not mixed, but exclusively of the one kind or the other, giving rise either to all green or all pale prothallia. Since here there can be no question of missing spores, the conclusion that segregation happens in somatic tissue, long before spore-formation, is inevitable. The character of the sporangia is, moreover, not determined by that of the area on which they stood, but was independent of it.

3. ROGUE PEAS AND ANISOGENY.

The genetics of rogues among culinary peas present two special features, of which one is so far without any known parallel. The facts are, in outline, that rogues arise spontaneously as a small percentage in many, probably all, modern strains of peas. The chief characteristics are *pointed* leaflets, an *upward curve* in the pods, and *reduction in size* of the appendicular parts. In most varieties the rogues are approximately uniform. *Gradus* is exceptional in having intermediates as well as the ordinary rogues. Rogues always breed true to their character. The *Gradus* intermediates throw *chiefly* rogues, with a few intermediates and an occasional type. Crosses between types and rogues of the same

¹ See *Journal of Genetics*, XIII. 1923. The work on *Scolopendrium* is still unfinished.

variety¹, however made, give F_1 in their early stages intermediate, but turning at or below the first flowers into rogues, producing, that is to say, rogue-like parts at and above these levels. Self-fertilised, these plants breed permanently true to rogue. We have been engaged on this investigation for some twelve years. In our opinion all we have seen is consistent with the supposition that the type-element is left behind in the lower parts of the cross-bred plant. In the *Gradus* intermediates the type-element commonly disappears more gradually. The change is generally marked at the level of the first flower, and progresses rapidly, as successive nodes are formed, so that the upper part of the plant is thorough rogue. Less often, the transformation is delayed and occasionally the pointed leaves do not definitely appear at all, though the pods curve. The genetical properties of these two classes of intermediates are different. The first group, as stated above, throw mostly rogues, with a few of the higher kinds; the incompletely transformed throw mostly types and high intermediates, but a percentage of true rogues greater than that thrown by real types. The progressive transformation at successively higher levels agrees well with our interpretation.

The genetical behaviour is also confirmatory. We have found by observing the produce of the several nodes self-fertilised, that the few types which the pointed intermediates give, come predominantly from the lower nodes. Above these there is an ordinal decline in the proportion of types produced at successive nodes. Just as the somatic character progressively changes, so—though the correspondence is irregular—the genetical properties follow. We have never seen any similar progression in the genetical behaviour of those intermediates which do not acquire pointed leaves. The rogues which they throw do not come with any special frequency from the higher levels, but are distributed uniformly among the pods, just as from the somatic appearance of the parents might be expected.

Finally, the pointed intermediates show also a progressive anisogeny. By reciprocal crossing between their successive flowers and types it was proved that the increase in rogue-production is very much more rapid on the male than on the female side. Miss Pellew has lately shown (unpublished) that even in an F_1 from type \times rogue (from a distinct variety), the lowest flowers fertilised by pollen of type may produce actual types. Nothing comparable has ever resulted from the use of F_1 pollen.

¹ In Sugar-pea crosses the intermediate nature may persist longer, so that the plants then pass for intermediates, and the same is occasionally the case in F_1 made from the cross of types and rogues belonging to very distinct varieties.

This series of facts taken together amounts nearly to proof that the somatic divisions result in the exclusion of a parental contribution, and that this process is orderly. By what cytological changes the result is accomplished we cannot surmise. It should however be remarked that in the organs of F_1 plants mosaicism occasionally exists—especially leaves having leaflets or half-leaflets of type and rogue associated; and that in *Gradus* intermediates these mosaic combinations are frequent, more particularly in leaves at the lower flowering nodes. We incline therefore to suppose, not so much that any successive cell-divisions result in progressive loss of the type-elements—which is most difficult to conceive—as that at some divisions, perhaps several, especially those associated with flower-formation, this element is dropped out. To obtain positive cytological evidence on such a point seems almost impossible. All that we know at present is that types and rogues do not differ in chromosome-number, both having $n = 7$.

4. CHIMAERAS, REGULAR AND IRREGULAR.

Ever since Baur demonstrated the fact that variegated periclinals give offspring of the type present in the subepidermal layer the great significance of this class of plants for genetical study has been recognised. Whatever be the true account of their germinal origin, here are plain instances in which the somatic differentiation can be often demonstrated to be a true indication of genetic potentiality. The application of such evidence to the general theory of heredity is open to the obvious objection that since the distinction between the layers is one of plastid physiology, the segregation of which these distinctions are the result may be wholly operating with the plastids themselves, whereas the segregations determining ordinary heredity operate with other elements of the cell, notably the chromosomes. Variegation, however, may come about from any of several dissimilar causes. It may be due to the inheritance of defective plastids or it may be a consequence, as Baur first showed, of the presence of a positive bleaching factor. This latter class of variegation is transmitted in ordinary Mendelian fashion; and though in none of the periclinal chimaeras where *white* is combined with green has the distinction yet been shown to belong to the Mendelian group, nevertheless those periclinals which have an *aurea* skin over a green core are a clear illustration of a somatic segregation in respect of the Mendelian bleaching factor. As Noack has seen and we here also have found, such periclinals breed as ordinary heterozygotes; and that the bleaching factor is restricted to the skin is proved by the fact that the green core, when it emerges,

breeds as an ordinary green. Therefore a Mendelian element is, by some process of somatic segregation, relegated to the skin. Evidence of the existence of certain analogous periclinals will be next considered. In dealing with variegation of the green parts the distinction between the outer and inner layers is plain to the eye on simple inspection, but by raising adventitious buds proof may sometimes be obtained that plants, not ostensibly chimaeras, do in fact contain a core having distinct characteristics. In a previous paper¹ I have described some of these in *Bouvardia* and in *Pelargoniums* of the fancy or "Regal" classes. The adventitious buds were in these instances obtained by growing pieces of roots removed from the parent plants. By this method the composition of a considerable number of varieties has been tested and in a few of them positive results have again been obtained.

In *Bouvardias*, *Oriental Poppies* and *Phloxes* root-propagation is so easy that the method is, or was, in common use among gardeners for commercial purposes. With other plants the amputated roots often rot without budding. Following the suggestion of Mr Stewart, of Edinburgh Botanic Garden, we tried lifting the plants so that the roots lay partially above the soil, and had some better success. This led to the bolder step of lifting the plants so that the roots were wholly out of the ground, cutting off the tops altogether a few inches above the collar, and keeping the whole damp under hand-lights on the fibre-bed in the stove house. For convenience of handling the whole beheaded plant is inverted and stuck stem downwards into a pot of soil, with the washed roots in the air. Frequent syringing gives sufficient water if the plants are kept close and moist. With many *Pelargoniums*, both *Zonal* and *fancy*, *Nicotianas* and various other plants, abundance of buds sometimes arise on the exposed roots. These root-buds, when they acquire a few leaves, are eventually cut off and encouraged to strike. At this stage many are lost, but a good proportion eventually root and become plants.

Bouvardia. As previously published, *Bridesmaid*, pinkish white double, has uniformly given *red* (Hogarth) double from its roots. These propagated again from roots have given their own type, but as an exception once, a *single red*. This latter has always given its own type only from its roots.

Vulcan, deep red, besides many like itself, gave once a rose pink.

Princess of Wales, a pinkish white single, the same colour as *Bridesmaid*, has, like it, given red singles from roots. At first I supposed that *Princess of Wales* must be merely a single *Bridesmaid*, but the leaves of

¹ *Journal of Genetics*, vi, 1916, and xi, 1921.

Princess of Wales and those of its ordinary root-“sport” are glabrous, whereas those of Bridesmaid and Hogarth are covered with short hairs. Since the anthocyanin is, as usual, confined to the epidermis of the petals, periclinal heterogeneity would not be *a priori* expected to be an attribute of any particular colour, and the coincidence is therefore remarkable.

Pelargonium. As already published, the named varieties of Fancy Pelargoniums known as Pearl, Mrs Gordon and Escot have all given distinct forms from their root-cuttings, the distinction in each case being primarily in flower colour. The flowers of Escot’s root are in addition flat and considerably larger than the flowers of the type, the petals of which are slightly rolled back. Similarly from Duchess of Portland, a *whitish* salmon-scarlet, the root gives a densely coloured flower of the same pigmentation, with guide-marks of increased size.

In three Zonals the roots have given plants with flowers differing in the development of the reproductive organs and in other respects. Salmon-fringed, in which the type has petals lacinated, with the female parts undeveloped, gives a normally hermaphrodite plant with normal petals from its roots. The leaves are also flat, not crumpled as those of the parent plant are.

Double New Life has very peculiar double flowers, female only, and devoid of anthers. Mr R. J. Chittenden has found it to give a normal hermaphrodite Zonal from its roots.

Kleiner Liebling, with minute, white-edged petals and no anthers, also gives a normal hermaphrodite with flowers of ordinary size from its roots. In contrast to these positive results, one or more root cuttings have been raised from 56 named types (miscellaneous fancy¹, Zonal, or scented-leaved) without giving anything different from the parent plants.

In all the examples hitherto given the inner component may be regarded with some plausibility as differing from the outer in the possession of a dominant. The only instances I have seen in which the converse arrangement appears to exist are certain purples or magentas. For example, Don Juan, a full magenta Fancy Pelargonium, was first known to me as a large plant at Kew bearing two kinds of flowers, some magenta, some crimson red, with others patch-works of both colours. When separately propagated the roots of the red form have always given reds, whereas the only two root-cuttings raised from the magenta have both been reds. Red may therefore be taken to be the inner component, and

¹ Many names, such as fancy, show, regal, etc., are applied to the large-flowered Pelargoniums, but I have not attempted to distinguish these.

judging from analogy is probably the recessive. The red Don Juan is liable to have occasional flakes of magenta, though the magenta has never in our experience had flakes of red. Whether this point is of significance I cannot say, but comparable instances have occurred with other reds and magentas in *Pelargonium*. Clorinda, for example, is a pink scented-leaved variety, which is also liable to have mauve flakes. From it we have raised a mauve sport by propagating a shoot which no doubt arose from a growing point in a mauve area. Root-cuttings from both the mauve and the pink give pinks only, though, as in Don Juan, these pinks are liable again to have mauve flakes¹. Rollisson's Unique is in all probability a comparable example, but beyond seeing purple trusses and flakes arising on the original red I have not yet tested it.

Besides these strikingly distinct plants which arise with some constancy (so far as observed) from roots of their respective types, a group of three root-cuttings from one Fancy seedling, bred here, contained two like the parent and one quite distinct in flower-colour. Evidently, therefore, uniformity in the distribution of characters among the somatic parts even of a single plant must not always be expected, and the analogy of chlorophyll distribution in the variegated plants is probably a true guide. Just as in the variegated plants rearrangements of the green and white parts are effected, sometimes with great frequency, by the divisions of the growing point, one or other of the components being often excluded altogether, so may similar changes happen in these periclinals. It is to be remembered also that in plants like *Pelargoniums*, which are continually propagated by cuttings, the original root is no longer present, and there is no certainty that the plerome of the stem is of the same genetic constitution as the original root. Experience of variegated plants shows also that it is from the *bases* of propagated shoots that stems showing new combinations especially appear, though *why* novelties should arise with special frequency in that position we do not know.

Of the varieties mentioned as having given novelties from their roots, Pearl, Salmon-fringed and Double New Life have shown more or less frequent sports or mosaics of the inner component, but neither Mrs Gordon, nor Escot, nor their root-cuttings, have ever given a spontaneous sport in our experience. These various degrees of constancy are exactly what we have observed in the behaviour of the several variegated types.

¹ The mauve form of Clorinda is known to horticulture as Joan. Whether the pink is the older form I do not know, but Don Juan traditionally arose as a sport of Lily Krumholz, which presumably was the red variety, though spoken of in catalogues as "rosy magenta."

Attempts have been frequently made to raise new plants from the leaves of *Pelargoniums*. Most commonly roots are formed from the callus at the base of the petiole, and such rooted leaves may live for months or even years without the production of any bud. From Escot on three occasions leaves have given buds which developed into plants. From such adventitious buds we expect the inner component to appear, and from two leaves plants did arise identical with those from Escot's root. The third leaf, however, gave a curious and interesting result. Three several shoots arose from it. One was the normal Escot, like the parent; another was Escot's root-form; and the third was distinct from either, having the size and markings of the root-form and the ground colour of the type.

Allocation of the characters among the somatic layers is not generally possible. In such a plant as Salmon-fringed we are clearly safe in speaking of the arrangement as periclinal; but though the constant appearance of a particular type from root-cuttings must indicate that the arrangement is orderly and doubtless that the root-form is the inner component, the structure of the variegated plants warns us that we cannot tell through how much of the plant that inner component extends. Further, such evidence as that from Escot's leaves shows that some recombination may take place in regions of active meristematic growth, and suggests that the process may not be, in any simple sense, orderly.

In view of Noack's¹ studies a doubt must be expressed whether any of these plants are periclinal chimaeras in the original implication of that term. We have hitherto supposed that the segregation to which the distinction between the layers is due had been determined in embryonic development, when the *plerome* was defined from the *periblem*. Noack comes to the conclusion that the outer and inner components owe their distinction to segregation continually recurring in meristematic divisions of the growing "labile" tissues. To decide between these two possibilities is not easy, but undoubtedly Noack's view has the merit that it somewhat diminishes the difficulty of imagining a mechanism by which changes in the relative positions of the layers can occur during vegetative growth. Such changes are sometimes frequent, as in *L'Élégante*, an ivy-leaved *Pelargonium*, commonly white-over-green, but always liable to produce green-over-white parts. The reversal may not infrequently occur in such a way that part of a

¹ *Verh. Phys. Med. Ges.*, Würzburg, 1924; *N.F.* XLIX. p. 45, and *ibid.* L. p. 47, 1925, and *Jahrb. wiss. Bot.* LXI. 1922.

leaf may show the white *outside*, the rest of the same leaf being green-over-white. In salmon-fringed *Pelargonium*, flowers are often patchworks of the outer and inner components, and on *Kleiner Liebling* we lately had a truss bearing several sterile flowers of the type on one side, and on the other side fertile flowers showing the character of the inner component in various degrees of development. In comment the similar production of patchworks in *Cytisus Adami* and the *Crataego-Mespitus* combinations may certainly be appealed to, but we know so little of the real nature of those plants that testimony derived from them has as yet no final validity.

Doubt may also be felt as to whether the root is in reality a periclinal combination in any of these plants. Up to the present we have no proof that the outer component extends into it. Sometimes it may perhaps do so, but the distribution of the green parts at least in Freak-of-Nature *Pelargonium* and in the variegated *Spiraea Ulmaria*¹ suggests that the root is wholly albinotic in those plants, and probably the same may be true of real periclinals.

Bud-sports have usually been regarded as evidence of a sporadic and fortuitous variation, there and then occurring in a somatic division. Question now arises as to whether they are not in reality the emergence of a constituent congenitally or at least long previously present in the plant. In practice, doubtless, both possibilities are realised, but I anticipate that in general, sporadic and spontaneous bud-sporting, as popularly conceived, is a very rare and exceptional event.

The genetical significance of somatic sports, whether sporadically emerging or arising from adventitious buds however produced, can only be determined by breeding. *Bouvardia* and *Pelargonium*, with which I have been specially concerned, are shy breeders and unsuitable for such determinations. As Baur showed in regard to the chimaeras of variegation and as Winkler's "graft-hybrids" between Tomato and *Solanum nigrum* also demonstrated, the genetic possibilities are those of the sub-epidermal layer.

Clausen and Goodspeed² have described two most interesting bud-sports in cross-bred *Nicotiana tabacum*. In regard to one of these the evidence was exceptionally complete. The plant was raised as F_1 from *N. purpurea* (carmine), fertilised by Cuba variety (white). F_1 is normally carmine, but one plant produced a pink-flowered sector. Both parts, the carmine and the pink, were in genetical composition qualitatively

¹ For figures of these plants see *Jour. Gen.* xi. 1921, Pl. XIV, figs. 2 and 3; xvi. 1925, Pl. IV.

² *Genetics*, viii. p. 97, 1923.

identical, giving carmines, pinks, and whites in somewhat irregular ratios. Each sector was then established on its own roots, after which the roots were propagated. The root-cuttings from the pink sector came carmine, showing that the deviation to pink was due to somatic variation affecting the epidermis only, the inner component being still the same.

As to two only of the chimaeras here discussed have we anything approaching evidence as to genetic constitution. The first is the Salmon-fringed Zonal Pelargonium. The outside of this plant is functionally male only and the petals are laciniated. Chittenden, using its pollen, was successful in fertilising the eggs of the inner component, and from the normal F_1 plants produced, raised F_2 . All the F_1 were normal and on being again bred with their own pollen gave a small F_2 generation, about 6, all normal and hermaphrodite, from which the inference may be drawn that the peculiar Salmon-fringed character was not introduced into the cross and was presumably a feature proper to the epidermis only¹.

The experience with Double New Life was more conclusive. Dr C. J. Bond was successful in fertilising the inner, normal, component of this variety with pollen of the abnormal external component. Chittenden repeated this, and, from the normal F_1 plants, raised about 90 plants in F_2 , all normal. Hence we may conclude with some confidence that the genetical peculiarity of Double New Life, viz. the doubling and the curious whitish intercalated petals², is present in the epidermis only and is not carried into the sub-epidermal layer.

A close study of the phenomenon of bud-sporting in plants may be expected to extend our understanding of the nature of the process of segregation and the principles which it obeys. I notice a disposition among the advocates of the chromosome theory in its cruder form to speak of all such sports as "mutations." Such an application of this term, for example, to the manifold sports which arise through the instability of any of the numerous arrangements of the components in a variegated plant is likely to introduce confusion. A branch of a new type may arise whenever one only of the components fortuitously comes to occupy a growing point, or by various rearrangements of the com-

¹ In the total sterility of Kleiner Liebling, and especially in the female sterility of Salmon-fringed and the male sterility of Double New Life, the two latter attributable to the presence of a special epidermis, we are reminded of Winkler's "*Solanum Koelreuterianum*," which having an epidermis of *S. nigrum* over a core of *S. lycopersicum* was likewise totally sterile. Winkler, H., *Zeits. f. Botanik*, Jg. 2, Heft 1, p. 16, 1909.

² Mr Chittenden has pointed out reasons for supposing that these whitish petals may represent the stamens.

ponents, to mention only the simplest possibilities. Mutation is a term used in a great variety of connotations by various writers. To disentangle these, even if it were possible, is a task beyond my present purpose, but in general I notice that the term is commonly employed to give an importance, even an evolutionary significance, to a change for which the common word variation is felt to be an insufficient description. To the consequences of rearrangement in a variegated plant such an expression is scarcely applicable, and in so far as a bud-sport may be merely the emergence of a pre-existing component no question of mutation arises, even though the emergent member may have distinct genetical properties.

The genetic nature of mosaics and sports is as yet obscure. In plants the problem has most commonly been investigated with reference either to variegation of the green parts, or to some feature of anthocyanin coloration manifesting similar properties. Inferences drawn from the genetics of variegation are of very doubtful application to other phenomena of heredity, for there are frequent suggestions that sometimes at least a segregation happens in respect of the plastids themselves. Moreover under the term variegation also a great diversity of conditions is included, which are alike chiefly in so far as they exhibit a common symptom. Some are simply infectious diseases, as may be seen in *Abutilon Thompsoni* and *Begonia manicata variegata*, where the variegation can be directly communicated by grafting. With these the geneticist is not immediately concerned. But even among the conditions which are unquestionably transmissible in heredity no one system of descent is followed. A few simple cases in which real variegation breeds quite true may at once be distinguished. In those with which I am personally acquainted the evidence indicates that the variegation is a simple recessive to green. The example most fully studied is the variegated *Tropaeolum*¹, the leaves of which show an intimate mixture of green and albinotic tissues. This variety was formerly the subject of extensive investigation made by Miss Gairdner in Cambridge and by ourselves independently at this Institution. We found it to behave as an ordinary recessive, which could be introduced from either the female or the male side of the parentage. An account of experiments with the same form was lately published by Correns², whose experience agrees with ours.

¹ For an example largely comparable though presenting certain differences, see I. Andersson, "Inheritance of Variegation in *Barbarea vulgaris*," *Journal of Genetics*, xiv. p. 185, 1924.

² *S.B. preuss. Akad. Wiss.* vi. 1920.

Like him we had expected green plants to arise from the self-fertilised seed of variegateds, but this never happened, variegated alone appearing. On one occasion however he had a green branch which developed on a variegated plant, and on self-fertilisation this proved to be heterozygous. Apart from this nothing exceptional occurred, and we have to recognise the paradox that the germ-cell of the variegated, whether male or female, is able to transmit the mixture of green and white cells, being itself mosaic in that respect.

As to the consequences to the heterozygote of the union of a real green-bearing gamete with one devoid of green we note that no rule can be predicated. The green may behave as a dominant, as has been seen by many of the experimenters with cereals. Sometimes the maternal character exclusively appears (e.g. *Antirrhinum* white-skinned fertilised by green), but not rarely the result seems to be almost fortuitous and governed by no system. As Baur found for instance in *Pelargonium*, and Chittenden¹ has seen in similar material, an irregular somatic segregation may occur in such heterozygote tissues, such that one or other component may be wholly or partially excluded.

In our present inquiry however we are primarily concerned with the gametic product of plants which certainly contain both elements and with the manner of their segregation. Baur first called attention to the difference in genetic behaviour between the sectorial and periclinal chimaeras, the former being irregular in their products, the latter regular. Variegation supplies several especially clear illustrations of this important distinction. Not merely the periclinals show a regularity in their genetic products, but some also of the variegated monocotyledons, in which an orderly striping, whether of white or green, occurs. In the periclinals we see a somatic segregation such that the genetic products agree in character with that of the foliar sub-epidermis. Similarly in striped *Chlorophytum* Collins² showed that it is the character of the central band of the leaf which is transmitted by the germ-cells. I have seen a similar example in *Funkia*, from the narrow white-edged variety of which green seedlings exclusively result. The yellowish white-edged *Sansevieria* common in greenhouses would doubtless show the same behaviour³.

The regularity or irregularity of the genetic behaviour of the varie-

¹ Chittenden, R. J., *Journal of Genetics*, xvi. p. 43, 1925.

² Collins, E. J., *Journal of Genetics*, xii. p. 1, 1922.

³ Propagated from leaves, this plant gives all greens, with no lateral bands, since the bud-forming callus is developed only from the green central band.

gated plants is evidently largely determined by the geometrical distribution of the chlorophyll character in the zygote. The geometrically regular arrangements have commonly an orderly genetic behaviour and the irregular mosaics have not. This distinction is largely independent of the nature of the character concerned. Provided that a character is *distributed according to some geometrical system governed by the normal differentiation*, it may be regularly transmitted; if the distribution shows no geometrical order, regularity in transmission is exceptional. Of this principle the genetics of variegation supply only a special case. It is probably of wide application in plant-genetics. We do not expect the anthocyanin striping of a flaked or bizarre Carnation to breed true, though the pattern of a wire-edged picotee, involving the same pigment, may be fixed.

One very remarkable example bearing on these questions, though it has other bearings also, may be introduced in this connection. *Myosotis*, "Star of Zürich," is a form having a definite white stripe down the centre of each blue petal. So far as I know its breeding had never been tested, and in horticulture it is always propagated by cuttings. Isolated in our breeding house it once produced seeds, which when sown gave exclusively plants with *white* flowers. I tried on many occasions subsequently to self-fertilise it or to make it cross with some other variety, but without success. In the open, exposed to insect pollination, it gives plenty of seeds, but these, neither in the first nor in any subsequent generation, have ever given the Zürich flower again. R. J. Chittenden at length, with considerable manipulative skill, succeeded in fertilising Star of Zürich with pollen of white flowered forms and thus raised many seedlings *all with white flowers* which have bred true. White is recessive in *Myosotis* and we thus have proof that in some way the germ-cells carry the character of the white central stripe and not that of the rest of the corolla. At present I know no clear parallel to this example of a somatic difference in anthocyanin development corresponding with a genetic distinction.

Irregular Mosaics in Plants and Animals.

No attempt to represent analytically the genetical behaviour of irregular mosaicism in plants has been altogether satisfactory. Confusion has arisen from the fact that a few mosaics are genetically real recessives, like the variegated *Tropaeolum* mentioned above, whereas most are of an altogether different nature. As regards mosaics in anthocyanin development I cannot recall any example in which a strain

breeds true in respect of a wholly irregular distribution. Plants with irregular stripes or specks of anthocyanin are, I believe, always liable to throw self-colours with greater or less frequency.

Since usually the pure self crossed with the mosaic gives F_1 self-coloured, the relation has often been represented as that of an ordinary dominant and recessive. The subsequent history of such families is then that the self, though often throwing the mosaics, can eventually be bred true. The mosaics on the contrary behave irregularly, and with greater or less frequency throw the selfs again. This behaviour, which is evidence of some residual difficulty, is sometimes referred to "mutation," surely a misnomer.

Avoiding such expressions as genes for self-colour or genes for mosaicism, the phenomenon may, without any violation of common experience, be represented in quantitative terms. The self-colour being taken as a unit, if a complete unit be introduced on fertilisation by either gamete the result is a self. If either gamete contribute an incomplete unit to F_1 , mosaics result in F_2 . If both gametes contribute incomplete units the result is a mosaic soma; but by segregation in the somatic divisions of such mosaics, cells, groups of cells and tissues *may* generally be formed which contain the unit again in its entirety. We do not know enough of the mechanics of these processes to justify any choice of an analogy, but provisionally I think of the separation-out of the unit as like the running-up into drops of the globules in an emulsion, though I am unable to symbolise that conception in terms of chromosomes. The genetical behaviour seems clearly to indicate that if, as one might expect, the unit can be restored to its quantitative integrity and completeness for any somatic cell, then in plants, at all events, quantitative gametic purity can ensue¹.

I am tempted to regard Mendelian segregation in general as a process not essentially different from that which we see working in the mosaics. It is the settling-out of an element *quantitatively unimpaired*, from a combination in which, but for this property of segregation, this element would be diluted.

¹ One of the best accounts of the genetics of a mosaic plant is that lately published by Eyster ("A Genetic Analysis of Variegation," *Genetics*, ix. p. 372, 1924) on the striping of the pericarp in maize. It should be noted that this striping is a phenomenon of anthocyanin distribution, which is not what we call variegation—a term here restricted to miscellaneous chlorophyll defects. The paper supplies an abundant array of facts and especially some welcome evidence bearing on the difference in genetic behaviour between the finer and the coarser mosaics. The discussion, though employing a terminology open, as I think, to criticism, is far more illuminating than those commonly supplied.

Admittedly the process is usually postponed to the reduction-division. Perhaps in animals it is generally so postponed. Of mosaicism in animals we know very little. In the animal examples which are clearest the mixture is in respect of sex-characters, and we may be well content to see, with Morgan, in such examples a suggestion that some sex-chromosome or one of its constituents may be there mosaically distributed in the somatic tissues. Apart from sex-mosaics, the phenomenon so common among plants¹ is singularly rare in zoological material—a contrast which in any consideration of the difference between plants and animals should be remembered. Nevertheless in one remarkable example of frequent mosaicism, the intergrades between the “species” of *Colaptes*², where certainly the mosaicism is not a matter of sex, the multifarious distinctions can only by a severe strain on the imagination be attributed to the abnormal distribution of a single chromosome. As to the genetics of mosaic animals experimental evidence scarcely exists, nor have we any material likely to supply it. If the broken colours characteristic of so many breeds of animals were to be found on plants we might with great confidence expect them to be an indication of mosaicism and to be genetically unstable, but the geometrical relation of the animal to its germ-cells is not like that of a plant. Perhaps the colours of Great Danes and of some Collie breeds, in which heterochromic eyes are common, may be mosaic in the genetic sense, but that is by no means certain. All we can see is that though a mosaic distribution of the factorial elements in the somatic tissues of a plant may and often must be an actual plan of the distribution of the several types of germ-cells, no such relation between germ-cells and soma can exist in any simple animal in which the germ-tract is early set apart.

We are reminded of mosaicism by the curious results obtained by

¹ The great collection of records relating to bud-sports of all kinds is that of Cramer, P. J. S., “Kritische Übersicht der bekannten Fälle von Knospensvariation,” *Natuurk. Verh. Holl. Maatsch. d. Wetensch.*, 3e Verzameling, Deel vi. 3e Stuk, Haarlem, 1907. Those who have no horticultural experience, who refer to Cramer’s work, will appreciate what a conspicuous and frequent part somatic segregation plays among garden plants, attributable without doubt to their heterozygous nature. Incidentally Cramer gives many examples of distinctions in alleged root-cuttings, but in many of these, especially those so commonly witnessed in the propagation of Chrysanthemums, we must not in the absence of anatomical examination assume that the sources of the new shoots were structurally roots.

That the great majority of illustrations relate to colour-variation is presumably attributable to the facility with which colour changes attract attention. Structural changes of the same class are no great rarities. For striking recent examples see I. Andersson’s work on *Barbarea* (*Journal of Genetics*, xiv. 1924).

² See *Problems of Genetics*, p. 150, 1913.

Bagg¹ and Little in producing, by X-rays, an injury to the germ-cells of mice, such that microphthalmus resulted in one or *occasionally* in both eyes, behaving as a recessive character. Later it was also discovered that a defect of the kidney was commonly associated with this lesion. We may have here something analogous to the condition of the germ-cells in, for example, the variegated *Tropaeolum*, which breeds true even though the somatic feature is a patchwork, and in segregation the "pure" is not reconstituted. Though exceptional in plants, this behaviour appears to be the rule among animals. The white Rosecomb Bantam, though always in our experience having a small coloured area, usually only two or three feathers, or even mere patches of colour on a few feathers, throws neither coloured nor white birds. The patch of colour is a definite constituent of the zygote which, on complete unpacking of the contents, comes to the surface. That this should be a normal genetical behaviour for a mosaic animal, though exceptional and somewhat unaccountable in a mosaic plant, is what the difference in the geometrical organisation of the two types of life leads us to expect.

The quantitative system of factorial analysis thus provides an adequate picture of the genetical behaviour of mosaic plants, in so far as it represents the properties of the two components after segregation, but the mode of that segregation still remains obscure. It might be conjectured that the mosaic cell has a mixed set of chromosomes which sort themselves out at random and thus reconstitute the two components pure, but this suggestion leads to difficulty as we shall immediately see.

Somehow a somatic cell is evidently able to divide in such a way as to produce cells dissimilar either from the parent cell or from each other, or both. The dissimilarity may be in the visible properties, as in differentiated tissues, or in such properties *and* in genetical potentiality also, as in the mosaics. In the progress of normal development we are familiar with divisions to which, so far as visible properties are concerned, the same description applies, and it is one of the paradoxes of cytology that, whatever the resulting tissue, its chromosome-content is the same, or at least shows no perceptible consistent distinctions; and—a phenomenon still more unaccountable—though, as I understand, visible distinctions between the chromosome-contents are not uncommon in somatic tissues, yet they are not specially associated with visible mosaicism. If we conceive the progressive differentiation by which the petal of a Carnation, for example, is developed we must admit that, by a series of cell-divisions, colourless epidermal cells and coloured epidermal cells are both produced

¹ Bagg, H. J., *Proc Soc. Exp. Biol. Med.*, xxi. pp. 146 and 228, 1923-4.

by division from a common origin, whether the resulting flower is bizarre or a picotee, and yet arguing from genetical properties we should have to believe that the distinction to which colour must be due is in the one plant a consequence of cytological difference and in the other plant that it is not. The dilemma is obvious.

Let us look at the alternative interpretation. The substances *seem* to be identical in the two plants. I should take this appearance as provisionally a reliable guide. The perceptible distinction between the mosaic bizarre and the differentiated colour of the picotee is one of configuration. Following that clue I anticipate that the solution is to be found not in any search for a distinction between the materials but in an analysis of the forces distributing them. In so far as a character is mosaically distributed in these plants, the very material, which might be treated as distributable according to the geometrical plan of normal differentiation, has escaped from that control. Whichever be the true account, the solution is to be sought in a proper understanding of the nature of the distinction between differentiation and genetical variation.

Cytology is providing some knowledge, however scanty, of the material composition of the cell, but of the nature of the control by which a series of orderly differentiations is governed we have no suggestion.

At which of the somatic divisions a segregation can happen such as to produce mosaicism or a genetical distinction is quite uncertain, but the evidence shows conclusively that this phenomenon sometimes occurs, and that though it may be a sporadic event, it may on occasion exhibit features indicative of regularity.

In conclusion we do well to remember that in a few curious examples fertility is not attained until some special somatic segregation has taken place. Of these the Zonal Pelargonium, "Freak of Nature," is the most definite and remarkable¹. The plant has both stems and the *centres* of the leaves of a peculiar white which may eventually become irrorated with green. The margins of the leaves, however, are solid green, and being too large to fit the white centres they are thrown up into folds. In this condition the plant flowers but has abortive pollen which, though it goes through a reduction division, is so defective that the small anthers shrivel away without bursting. The female side is also sterile. But when by some somatic division either component separates from the rest, forming a green stem or a white one, these on flowering are functionally fertile.

¹ See Chittenden, R. J., *Journal of Genetics*, xvi. p. 48, 1925.

Having in view the various facts and considerations here enumerated I think we shall do genetical science no disservice if we postpone acceptance of the chromosome theory in its many extensions and implications. Let us distinguish fact from hypothesis. It has been proved that, especially in animals, certain transferable characters have a direct association with particular chromosomes. Though made in a restricted field this is a very extraordinary and most encouraging advance. Nevertheless the hope that it may be safely extended into a comprehensive theory of heredity seems to me ill-founded, and I can scarcely suppose that on a wide survey of genetical facts, especially those so commonly witnessed among plants, such an expectation would be entertained. For phenomena to which the simple chromosome theory is inapplicable, save by the invocation of a train of subordinate hypotheses, have been there met with continually, as even our brief experience of some fifteen years has abundantly demonstrated. Through all this work, with ever increasing certainty, the conviction has grown that the problem of heredity and variation is intimately connected with that of somatic differentiation, and that in an analysis of the interrelations of these two manifestations of cellular diversity lies the best prospect of success. Pending that analysis, the chromosome theory, though providing much that is certainly true and of immense value, has fallen short of the essential discovery.

CHROMOSOME STUDIES IN THE SCILLEAE.

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(With Four Text-figures and Four Plates.)

MATERIAL.

A CASUAL examination of root-tips of a triploid Hyacinth last spring showed features of interest relating to the form of the chromosomes which encouraged me to investigate other stages in detail.

Previous work has not shown very satisfactory agreement; Carruthers (1921) concluded that "The constriction of the chromosomes into separate segments is of common occurrence throughout mitosis," while De Mol (1921) definitely attributes the occasional constrictions that he has noticed to the process of cutting, thus: "...dans les cellules des racines de la variété Grand Maître il ne peut être question d'admettre ni l'existence de satellites ni la présence de chromosomes en nombre anormal"—"satellites" being used in a broad sense.

A diploid Hyacinth and several triploid varieties were studied during the autumn. Some bulbs of the variety "King of the Blues," passed through the second maturation division about the 27th October, others, having been planted in a cool frame meanwhile, had pollen actively dividing on the 20th November. The varieties "Queen of the Pinks" and "Grand Maître" were examined at the same time but bulbs of "Moreno," a pink flowered variety, "L'Innocence" and "La Grandesse," freshly arrived in sawdust, and diploid Hyacinths underground were going through this stage between 5th and 15th December.

Preparations were made from the root-tips of the following:

A common blue diploid variety.

Grand Maître.

King of the Blues.

Queen of the Pinks.

Moreno.

Marie (dark violet flowers).

La Peyrouse.

L'Innocence.

La Grandesse.

Totula.

I am obliged to Messrs James Carter and Sons for their gift of bulbs.

The material of *Bellevallia romana* was used up in premature attempts to obtain the pollen maturation divisions: later stages were found to occur when the spike was about to shoot (February 21st).

Preparations were made of the first division of the microspore of *Scilla nutans* which occurs directly before the appearance of the flower.

METHODS.

The fixative has in all cases been a modified Flemming mixture consisting of:

- 24 cc. 1 per cent. Chromic acid.
- 8 cc. 2 per cent. Osmic acid.
- 1 cc. Glacial Acetic acid.

All preparations have been stained with gentian violet, iodine being used to prevent undue loss of stain during dehydration.

Sections of Hyacinth root-tips cut 15 to 17 μ thick were very satisfactory. Anthers and root-tips of *Bellevallia* were cut 11 to 13 μ (and *Scilla nutans* root-tips 10 μ thick): longitudinal as well as transverse sections of the root-tips were made.

The vegetative divisions of the pollen grain were prepared as smears, the method used being more or less that described by Taylor. In the Hyacinths it proved quite unnecessary to bleach after fixing but smears of *Scilla* and *Bellevallia* were treated for half an hour with solutions of hydrogen peroxide in 80 per cent. alcohol.

The method is particularly successful with this material owing, to some extent, to the large size and fairly regular shape of the pollen grains. The anthers containing fully formed pollen-grains are a little drier than at an earlier stage so that a drop of water on the slide is useful, not rendering the pollen grains less adherent and enabling one to take a little longer in spreading—say 10 seconds—a flat surgical needle being used for the purpose. I did not find any derangement in the grains (which, of course, are less susceptible of injury than the pollen mother-cells) that could with certainty be attributed to the treatment. The fixations were highly satisfactory and the slight muddiness of two or three slides was, I believe, due to leaving too long in the stain.

One advantage of smearing is that the pollen grains may remain movable; thus an oblique metaphase plate may sometimes be turned over by tapping the cover-slip and be set perfectly flat; Fig. 20 is a drawing of a division so treated which could not otherwise have been

counted. Such grains may, of course, shift their position spontaneously, and in turning over become obscured.

For the methods employed I am indebted to my colleague, Mr W. C. F. Newton, whose advice and experience have been invaluable to me throughout.

HYACINTHUS ORIENTALIS: CHROMOSOME TYPES.

The chromosomes of *Hyacinthus orientalis*, which have been described as being of three types, fall into four distinct classes by the position and number of their constrictions (Text-fig. 1).

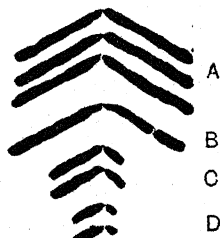


Fig 1

Text-fig. 1. Chromosome complement of *Hyacinthus orientalis*.

A. Three long chromosomes with a median constriction.

B. One long chromosome having one arm like an arm of A, and the other slightly shorter and constricted, forming a longer proximal element and a shorter distal element.

C. Two medium-length chromosomes, each shorter than one arm of a long chromosome and with a subterminal constriction.

D. Two short chromosomes each about three-fifths of the length of C, also with subterminal constrictions.

The complement may thus be represented as $3 + 1 : 2 : 2$. If, therefore, "doubling" has taken place in the history of the species it must have preceded the development of type B chromosome unless the "doubling" happened after a cross: in any case the phenomena of tetraploidy, discussed by Belling, would hardly be expected.

Chromosomes of the different types have been measured for purposes of comparison, the pollen grain divisions being the most suitable for this purpose. The separate elements of a constricted chromosome must be taken separately and added together, for a measurement including the variable width of the hiatus would be of no value. Thus it is that

the constricted arm of the odd long chromosome, though appearing very little shorter than the other arm, is really decidedly so.

Not improbably, different degrees of contraction may cause chromosomes of the same type in the same division to differ in length perceptibly at metaphase, and still more so, of course, at anaphase. The diagrams of the chromosome types are therefore given with reserve; they do no more than condense and confirm the impression obtained from the figures, from the best of which they are derived.

HYACINTHUS ORIENTALIS: GENERAL OBSERVATIONS.

Somatic Divisions. At metaphase in the root-tips no strict system is noticeable in the disposition of the chromosomes: their mass, at least in the heteroploid varieties, seems to be too great to allow of their fitting into one another in a restricted space in one plane. They are distributed through a greater depth than is usually the case. The longer ones especially are much contorted; recognition often requires familiarity with the typical shapes assumed and only occasionally are they flat enough to be measured reliably. Constrictions are always visible unless concealed by the bending of the chromosomes.

Although, therefore, fixations of this material were satisfactory it will be understood that somatic divisions cannot yield really accurate results in respect of chromosome size and individuality.

The chromosome numbers of two varieties, not previously examined, were counted and in each irregularities were noted, no doubt, owing to the root-tips growing in water. Moreno normally appears to be a true triploid but an extra short chromosome was undoubtedly present in one division. La Peyrouse has one long chromosome more than the triploid as a rule but divisions were counted with 14 long chromosomes, the other types being present in the normal numbers.

It was expected that plants having the highest number of chromosomes would be the most liable to show abnormalities of this type. Examination of Totula, a variety described by De Mol, showed different chromosome numbers in the same root-tip as to each of the several types and as to the whole complement. The tetraploid number of one of the types was apparently exceeded in some cases. Four divisions were counted with 30 chromosomes and three with 31 chromosomes. De Mol counted the chromosomes in the proportion 15 : 8 : 7. Fig. 5 shows the complement 16 : 6 : 8 and Fig. 6, 16 : 6 : 9 (all of the four long chromosomes with the second constriction being distinguishable). The occurrence

of nine short chromosomes—in excess of the tetraploid—has been noted clearly in other divisions in this variety. Cells of this constitution can therefore continue dividing although, perhaps, whole individuals would be incapable of developing from seed.

Fig. 2 shows a striking example of the development of secondary constrictions at anaphase as a result of the special state of tension of the chromosomes.

2nd p.m.c. division. The constrictions are generally less pronounced than in the somatic divisions. The degree to which the gap remains distinct depends on the position of the chromosomes and the phase of division; at early metaphase it may be rendered conspicuous by the tendency especially in the long chromosomes of the constricted regions of the daughter halves to remain in close contact, while the distal portions lie freely.

Fig. 7 is a lateral view of an anaphase showing two long chromosomes the fate of which still seems to be in doubt.

First Microspore Division.

Prophase. In spite of the approximately spherical space in which the chromosomes are disposed it is frequently possible to find individual chromosomes nearly flat. The constrictions which are normally marked at metaphase are then clearly seen, but other constrictions of less importance are distinct in the earlier stages. With the help of the constrictions it is often possible to recognise with moderate certainty to which type each chromosome belongs.

Fig. 25 shows five long, two medium (at 1 and 2 o'clock) and four short chromosomes. The ?-shaped chromosome has the median constriction and a second constriction in one arm. A secondary split is obvious in one of the chromosomes. Fig. 26 showing five long, two medium and two short chromosomes represents at a somewhat later stage the same ?-shaped chromosome with the second constriction.

Metaphase. The tendency of the chromosomes to lie with the point of attachment—a constriction—towards the middle of the plate, the larger chromosomes distributing themselves about the periphery and the smaller centrally, gives an appearance of regularity which often simulates “pairing.” That mutual adjustments in a restricted space are largely responsible for this result, as in the diploid divisions, is shown by its occurrence in haploid pollen grains and by the “pairing” of the odd long chromosome with one of type A.

Anaphase. An appearance of duality is given to chromosomes at

anaphase in favourable cases by vacuolation, by the undoubted lobing of the tip, particularly in long chromosomes, and also perhaps by the doubleness of the constricted region. In one case this condition was strikingly obvious at metaphase (Fig. 13). Taylor has recently remarked on the same premature appearance in *Gasteria*. It is hard to believe that these signs of fission are unconnected with the next real division of the chromosomes.

Segregation. Counts were made of the number of chromosomes taking part in divisions from prophase to anaphase: the various possible numbers occurred in the proportions set out in the Table. Fig. 11 shows the extreme case of a plate with the whole diploid complement.

These results agree fairly well with Belling's observations in acetocarmine preparations, quoted in the table for comparison. Belling noted that the excess of numbers below 12 over those above 12 in his counts was 7, *i.e.* 13.7 per cent. of the total number; here the excess is 20.7 per cent. (160 pollen grains between 8 and 11, 92 between 13 and 16) a discrepancy from the relation expected under random segregation which, in view of the large number of observations involved, is, I submit, far too great to be dismissed as due to error. We may therefore consider the action of certain influences which would give an expectation agreeing better with the results.

A differential viability amongst pollen grains acting against those with the higher numbers—analogous to the falling off which De Mols' observations show in the higher numbered plants as compared with the triploids—would partly account for the principal discrepancy. If we assume that the proportion of grains with higher numbers originally produced corresponded with the proportion of grains observed with lower numbers the original total would have been 397 grains. The 329 examined are thus the survivors from 397, of which the line B (in the table) gives the proportions expected with random segregation in the first division. It is not possible that differential viability alone could account for the relative losses shown by the figures observed, affecting, as they do, the middle numbers more severely than the higher.

The element of uncertainty in the second division (illustrated in Fig. 7) is sufficient to warrant our considering the effect of randomness in the distribution of the odd chromosomes at that stage; *C* shows the proportion that would be expected if the segregation were purely fortuitous: a slight degree of randomness such as we may reasonably surmise would produce a curve intermediate between *A* and *C*. Taking the figures of *C* as a proportion of 397 we have *D* which agrees well

with the results except in the number of pollen grains having 8 chromosomes.

The chance of loss of chromosomes during the maturation divisions is a third factor capable of influencing the proportion. *E* shows the numbers expected from random segregation in the first division, accompanied by a loss of extra chromosomes subject to definite and constant simple chance or combination of chances of 0.1. The agreement with the observed figures is generally satisfactory, except perhaps in the middle numbers in which the deficiency may very well be due to the effect of irregular segregation at the second division.

BELLEVALIA ROMANA.

Chromosome types. The chromosomes are all distinct in length as well as in shape, each being constricted so as to have the form of two arms. In the figures and diagrams they are denoted with letters thus:

"A" the longest chromosome has almost equal arms, the shorter having a terminal satellite. Fig. 19 shows chromosomes of this type with the longer arm having a second sub-terminal constriction; this is probably a constant state although neither so pronounced nor so regularly occurring as the second constriction in *Hyacinthus orientalis*.

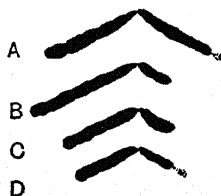


Fig. 2

Text-fig. 2. Chromosome complement of *Bellevalia romana*.

"B" has one arm longer than either arm of "A," the other very short, separated by a constriction.

"C" resembles "B," but the longer arm is shorter than in "B."

"D" is the shortest chromosome. Its shorter arm bears a satellite.

Text-fig. 2 shows the average relative lengths and the shapes of the chromosomes derived from drawings of somatic divisions.

General Observations.

Somatic divisions. Root-tips in transverse section showed divisions in which four types of chromosomes were discernible but the usual obliqueness of the long arms obscured their relative lengths. The number of chromosomes being few it was thought that in the side views of

anaphase from longitudinal sections it would be possible to distinguish the types more clearly: Figs. 18 and 19 are anaphases which have been moved sideways, between drawing successive chromosomes, so as to show them separately.

One constriction in each chromosome seems to be constant, and similar to those in *Hyacinthus orientalis*, but the satellite is a phenomenon not seen in that species. The satellite varies in appearance according to the differentiation, being either (i) stained as deeply as the rest of the chromosome, (ii) faintly stained and only noticeable on very careful examination, or (iii) not apparent at all, owing either to the staining being too faint, or to its lying directly over the main limb of the chromosome, or perhaps even to their being fused. The first two cases are, however, much too common to allow of any doubt as to the definiteness of the appearance.

2nd Microspore mother-cell division. Fig. 20 shows a second maturation division. No satellites are now visible and I consider it very likely that, at this stage, they are telescoped into the main body, even the principal constriction being indicated only by the angle of the chromosome.

SCILLA NUTANS: CHROMOSOME TYPES.

Eight types of chromosomes could be distinguished at the anaphase in diploid divisions corresponding closely enough with those seen at metaphase in the haploid divisions. At the diploid metaphase, however, while sometimes (Fig. 21) the chromosomes fall readily into the eight classes seen elsewhere, when due allowance is made for their contortions, at other times the types seem to follow another order. This may be due to the occurrence at prophase in the microspore division of strongly marked constrictions which can be accounted for at metaphase in these difficult cases but not at other stages; probably in the regular cases these constrictions are undeveloped or obscured giving the chromosome the normal appearance.

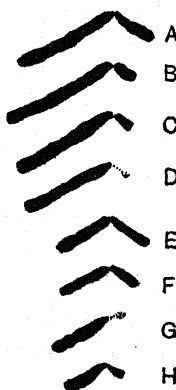


Fig. 3

Text-fig. 3. Chromosome complement of *Scilla nutans*.

The diagrams (Text-fig. 3) represent an average of the pollen grain and the somatic anaphase drawings (the somatic metaphase being useless for purposes of measurement). These show the metaphase appearance with no significant difference between "B" and "C" but at anaphase (Fig. 23) all four chromosomes of "C" are bent at an unconstricted point,

thus distinguishing it sharply from "B." The constriction separates only a satellite in "D," which has the same curious bend not exactly at a constriction.

SCILLA NUTANS: GENERAL.

Diploid divisions. The occurrence of extra constrictions at metaphase, particularly in "D" and "E" seems to be responsible for a difficulty in assigning each chromosome to its type at this stage. For example, the presence of an extra median constriction in "D" may cause that chromosome to be confused with "E," in which a second constriction is sometimes strongly developed. The anaphase was drawn in the same way as in *Bellevallia* (Fig. 23). Here, not merely the existence of eight distinct pairs is clear, but their relative sizes and shapes are accurately shown; each chromosome being sufficiently characteristic to indicate to which type it belongs, each type is confirmed four times. It will be seen that all four examples of "C" are bent at an unconstricted point which moves foremost to the pole.

Most interesting was the observation from a root-tip growing under normal conditions, of divisions having, some one, some two extra chromosomes. None were found with too few, because, doubtless, the nuclei with less than the diploid number fail to divide further.

1st Microspore division. Constrictions of varying importance can be seen at prophase (Fig. 27) as in *Hyacinthus*. It should be specially noted that five of the chromosomes have a roughly median constriction strongly marked whereas only one chromosome at metaphase can be said to have a constriction at all approaching median; although, therefore, it is impossible to assign each chromosome to a particular type at this stage we can suppose that a constriction apparently of primary importance at prophase may be suppressed at metaphase.

Some of the chromosomes show a marked secondary split, illustrated in Fig. 27. This was noted, though not so regularly in *Hyacinthus* (Fig. 26).

At metaphase the smaller limb of two chromosomes ("D" and "G") was sometimes of the satellite type (Figs. 23 and 24), sometimes like a pimple on the end of the chromosome (Figs. 21 and 22), deeply stained and in every respect resembling the small head of a constricted chromosome.

CONSTRICTIONS AND SATELLITES.

The point of discontinuity in the fine chromatin thread at prophase may be traced at successive stages until it becomes recognisable as a

"constriction" at metaphase. From the fact that this constriction may become the angle of the bent chromosome and move foremost to the pole, it has been argued (Newton and Taylor) that the constriction is necessarily the centre of attraction to the pole, or the "point of attachment" to the so-called spindle fibre.

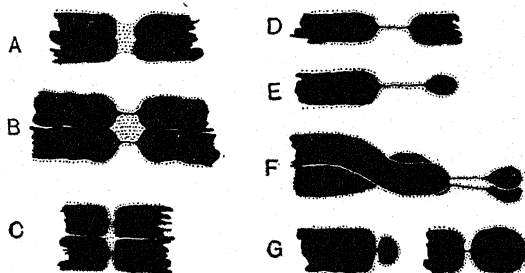
Chromosome "G" (Fig. 23) is seen to be approaching the pole with the satellite foremost: this means that the satellite is endowed with special responsiveness to the attraction of the pole; yet it can hardly be the sole seat of response for, were it so, the whole body of the chromosome would have to be drawn by the fine connecting thread which could hardly bear such a tension—certainly not without extreme stretching. Probably, therefore, the centre of responsiveness is not strictly localized.

Constrictions may commonly occur as we know at points quite unconnected with any centre of attraction and the centre of attraction may occur, as I have observed in *Scilla nutans*, at a point where there is no sign of constriction: in other words no causal relation need exist between the constriction and the centre of attraction and no specific reason can be alleged why either the centre of attraction should determine the existence of a constriction, or the constriction should determine the position of the centre of attraction.

In view of the large number of observations that have been recorded, all pointing apparently to the contrary, this conclusion may seem presumptuous, but I think there is a reasonable explanation of the disagreement. Responsiveness to the polar attraction is, we may assume, diffused over a restricted area of the chromosome; when the attractive force operates the chromosome will bend in this region, a sharp flexure occurring at any weak point, that is at a constriction if there is one close enough. Only, therefore, in the absence of a local point of discontinuity will the "point of attachment" appear to be unassociated with a constriction; thus an independent flexure of a chromosome, as in *Scilla nutans* may be quite uncommon.

In some divisions a chromosome may appear as though in a state of tension and then the hiatus, or rather, as I believe, the two threads joining the two elements of the chromosome will appear stretched; in other circumstances the chromosome may appear condensed and the constriction may be reduced to what is indicated by the name itself (Text-fig. 4, C and G). A bend in a partially split chromosome, as in "B" (Fig. 24), may simulate a contracted constriction. This is especially deceptive in the case of the satellite; where the two arms cross over one another there may appear to be a second constriction (Text-fig. 4, F).

The exact nature of the constriction is necessarily elusive. Text-fig. 4 shows possible interpretations. *A* represents two elements of a chromosome joined merely by an elastic film of faintly stained material—the supposed coat of the chromosome itself. *B* and *C* represent the condition when the constricted halves of a splitting chromosome are each joined across the gap by a thread of chromatin. *D* is a common and most definite appearance as a lateral view of the constricted chromosome; it could only appear as a form of *B* and *C* and points strongly to the connection being of a chromatin nature. The negative argument of the



Text-fig. 4, A-G. Diagrams to show possible interpretations of the appearance of constrictions and satellites.

frequent *absence* of any appearance of a chromatin thread is, I think, of little weight. A dark area subtending a small enough angle at the eye and surrounded by a light area, will appear diffuse and faint when seen by transmitted light. This illusion, which may be reproduced by viewing Text-fig. 4, B, out of focus of the eye, may account for the frequent difficulty of seeing the thread. It must also be borne in mind that the thickness of the thread is but a small fraction of that of the chromosome; before differentiation of the main mass has had any appreciable effect, therefore, the thread will be de-stained, while even if stained its thinness must render it paler than a bigger mass of chromatin.

The ends of a split chromosome at metaphase naturally lie more freely than the parts relatively proximal; we have, thus, the frequent appearance of the daughter chromosome adhering by a median constriction; at late metaphase, of course (Fig. 15), the polar attraction separates the responsive centres, while the distal portions remain closely attached.

The three species described have constricted chromosomes whose limbs range in size from the isosceles type of *Hyacinthus orientalis* to the satellite type of *Scilla nutans* where one mass may be hundreds of

times greater than the other. If one is to distinguish between the dependent satellite and the larger elements the only critical test to apply is one of size. There comes a point as the mass of an element decreases when the surface tension of the film, in itself tending to keep the element spherical, must reduce the width as well as the length; a portion thus narrower than the main body of the chromosome seems to require the name of satellite or *trabant*; such an element, having an attraction for the parent body proportionally less than a larger element, is naturally more subject to external forces, hence the common appearance of flying out (Text-fig. 4, *E* and *F*). In this connection it is noteworthy that a satellite may be so small as to come within range of size of particles subject to Brownian motion. In the absence of distractive forces the satellite will appear closely appressed to the parent body (Text-fig. 4, *G* and Fig. 21, chromosome "G"). The frequently fainter staining of the satellite depends on the same conditions which have been shown to influence the appearance of the chromatin thread.

Navashin's observation of the relationship of the satellite to the nucleolus in prophase has not been confirmed in these studies nor does any abnormal function or property of the satellite seem to be indicated at any other stage. I consider, therefore, that the satellite is the natural result of an extreme type of sub-terminal constriction.

SUMMARY AND CONCLUSIONS.

The chromosome types of three species, *Hyacinthus orientalis*, *Bellevalia romana* and *Scilla nutans*, have been examined at various stages; the inter-relation of the complements does not point to any affinity between the species or to any common descent from a three-chromosome ancestor. The complement of the Hyacinth is not capable of a purely tetraploid interpretation because one of the four long chromosomes has a definite and constant second constriction while the other three are alike in every respect.

The condition of the constrictions in the three species was followed at different stages: certain constrictions are permanent, others appear at one stage but may be suppressed at another. The phenomenon seems to be the result of a local discontinuity which occurs in different chromosomes at different positions—bisecting the chromosome or cutting off a minute element, which will form, in the contracted state, a "satellite." Reasons have been shown for believing that the responsiveness in the chromosome to the attraction of the poles is more diffuse than is usually

imagined and that this zone of response need not coincide with a constriction.

The appearance of the constriction in all cases may be accounted for by supposing that two chromosome elements are joined by a fine single, or double, chromatin thread surrounded, no doubt, by the film-coat that envelopes the whole chromosome.

Secondary splitting of the chromosomes in the first microspore division was noticed at all stages in *Hyacinthus orientalis*, and in *Scilla nutans* in prophase.

Counts have been made of chromosome numbers in the pollen grains of triploid Hyacinths: various theoretical explanations of the proportions in which they occur have been tested and the results have been found to agree closely with the assumption that each of the extra chromosomes undergoes a constant chance of loss, of the order of one-tenth.

The occurrence of somatic variations in chromosome number was noted in heteroploid varieties of *Hyacinthus orientalis* and in *Scilla nutans*.

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TABLE.
Counts of pollen-grain divisions in Triploid Hyacinth showing the segregation of extra chromosomes.

Nos. of chromosomes observed	Total	8	9	10	11	12	13	14	15	16
In "King of the Blues"	199	1	14	35	49	47	33	18	15	16
In "Queen of the Pinks"	55	1	1	10	10	14	10	5	2	—
In "Grand Maître"	69	1	8	11	16	16	11	5	1	2
In "Moreno"	6	—	—	2	1	—	1	2	—	—
Total observed	329	3	23	58	76	77	55	30	5	2
By Belling (in "Lady Derby")	51	1	5	3	15	11	14	3	—	—
A. Expected nos. assuming random segregation	329	1.3	10.2	35.9	72.0	90.2	72.0	35.9	10.2	1
B. Expected nos. (8-12) if as many pollen grains above 12 were produced as below	396.9	1.6	12.3	43.3	86.8	108.9	(<86.8)	(<43.3)	(<12.3)	(<1.6)
C. Expected nos. assuming that random segregation takes place during 2nd division as well as 1st	329	7.5	23.6	44.0	58.2	62.4	58.2	44.0	23.6	7.5
D. Expected nos. assuming differential rate of division and 2nd division random segregation	397.1	9.1	28.5	53.1	70.2	75.3	(<70.2)	(<53.1)	(<28.5)	(<9.1)
E. Expected nos. assuming chance loss of 0.1 of extra chromosomes	329	2.3	17.9	51.7	84.2	87.3	56.6	23.0	5.4	0.6

EXPLANATION OF PLATES XV—XVIII. .

All drawings were made at stage level, using an Abbé camera lucida, a Leitz 2 mm. objective (n.a. = 15) and a 15 eyepiece (except Figs. 25–27). Magnification 1270, unreduced.

Figs. 1–15 and 25–26, *Hyacinthus orientalis*; 16–20, *Bellevallia romana*; 21–24 and 27, *Scilla nutans*.

PLATE XV (1–6). Root-tip divisions.

- Fig. 1. Metaphase (diploid) $6 + 2 : 4 : 4$.
- Fig. 2. Anaphase, incomplete (diploid).
- Fig. 3. Metaphase (Grand Maître).
- Fig. 4. Metaphase (Moreno).
- Fig. 5. Metaphase (Totula) 30 chromosomes.
- Fig. 6. Metaphase (Totula) 31 chromosomes.
- Fig. 7. Anaphase, 2nd pollen mother-cell division (King of the Blues)

PLATE XVI. Vegetative division of the pollen grain.

- Fig. 8. Anaphase (diploid).
- Fig. 9. Anaphase (Queen of the Pinks) $6 : 3 : 3$.
- Fig. 10. Metaphase (Queen of the Pinks) $4 + 1 : 4 : 3$.
- Fig. 11. Metaphase (Queen of the Pinks) $6 + 2 : 4 : 4$.
- Fig. 12. Early metaphase (Queen of the Pinks) $4 + 1 : 2 : 3$.
- Fig. 13. Early metaphase (King of the Blues) $6 : 3 : 2$.
- Fig. 14. Metaphase (Moreno) $3 + 1 : 3 : 3$.
- Fig. 15. Late Metaphase (L'Innocence) $5 + 1 : 3 : 3$.

PLATE XVII.

- Fig. 16. Metaphase, root-tip.
- Fig. 17. Late prophase, root-tip.
- Fig. 18. Anaphase, root-tip.
- Fig. 19. Anaphase, root-tip.
- Fig. 20. Anaphase, 2nd pollen mother-cell division.
- Fig. 21. Metaphase, root-tip (16 chromosomes).
- Fig. 22. Metaphase, root-tip (18 chromosomes).
- Fig. 23. Anaphase, root-tip.
- Fig. 24. Metaphase, vegetative division of the pollen grain.

PLATE XVIII. Prophases vegetative division of the pollen grain.

- Fig. 25. King of the Blues $4 + 1 : 2 : 4 \times 3600$.
- Fig. 26. King of the Blues $4 + 1 : 2 : 2 \times 3600$.
- Fig. 27. *Scilla nutans*. $\times 2200$.



Fig. 1



Fig. 2

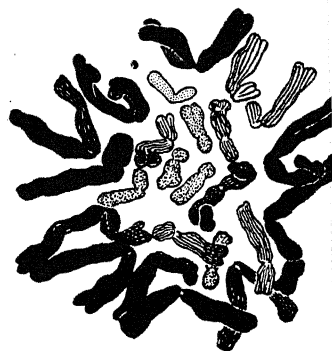


Fig. 3



Fig. 4

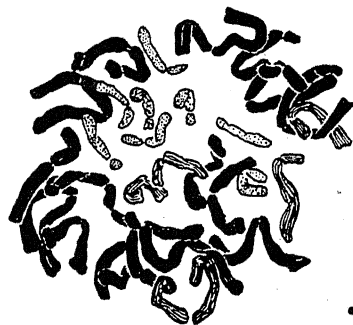


Fig. 5

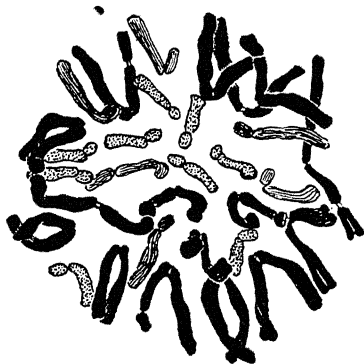


Fig. 6



Fig. 7

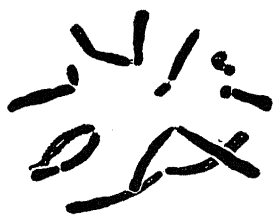


Fig. 8

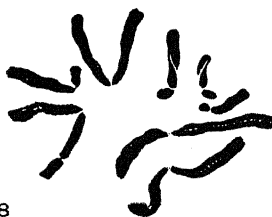


Fig. 9

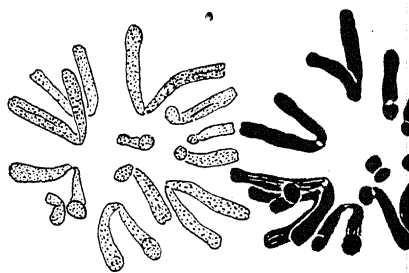


Fig. 10

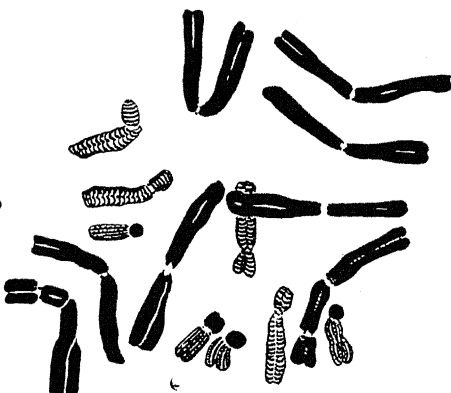


Fig. 11



Fig. 12



Fig. 13



Fig. 14



Fig. 15



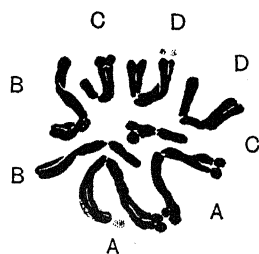


Fig. 16

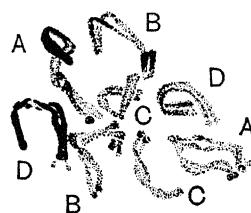


Fig. 17



Fig. 18



Fig. 19

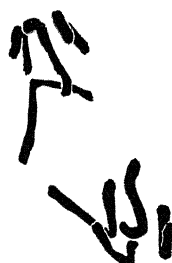


Fig. 20

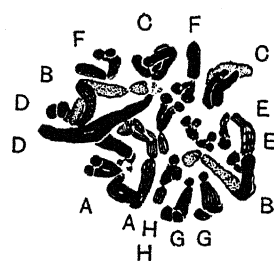


Fig. 21

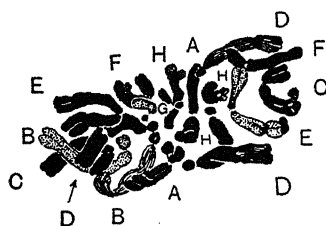


Fig. 22



Fig. 23

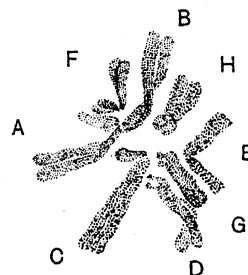


Fig. 24



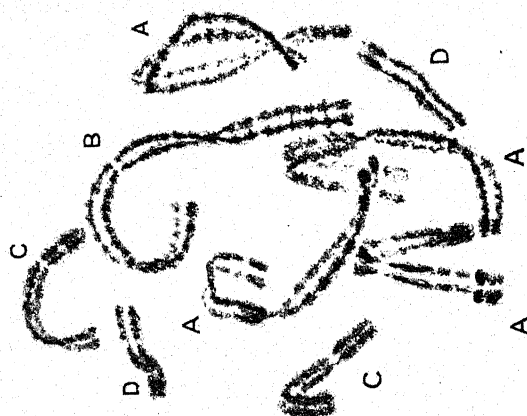


Fig. 25.



Fig. 26.



Fig. 27.

FURTHER OBSERVATIONS ON POLYDACTYLY AND HETERODACTYLY IN FOWLS.

By C. J. BOND, C.M.G., F.R.C.S.

(With Two Plates.)

In a former article (*Journ. of Genetics*, July, 1920), "On the left-sided incidence of the supernumerary digit in Heterodactylous fowls," attention was drawn to the fact that in a certain number of a-symmetrical birds—that is—birds with four toes on one leg and five on the other, there is a greater tendency for the extra digit to appear on the left side.

In this communication I propose to describe some of the results obtained by crossing the Houdan (five-toed) with the Andalusian (four-toed) breed from this digital aspect.

A first cross between an Andalusian cock and Houdan hens made in 1923 resulted in the usual preponderance of five-toed birds in the F_1 generation, together with a few a-symmetrical birds, among which the incidence of the extra digit was greater on the left side. In addition there were three birds having six toes on one foot and five on the other, and one bird with six toes on both feet.

In 1924 a number of these Houdan-Andalusian cross-bred birds of the F_1 generation were mated together.

In a total of 46 chickens of this mating there were 26 five-toed symmetrical, 14 four-toed symmetrical and 6 a-symmetrical birds. Of these latter two had four toes on the right and five on the left side, one five on the right and four on the left, and one five on the right and six on the left.

In 1925 one of these a-symmetrical F_2 birds, a black cock with five toes on the left and six on the right foot, was mated with six hens of the same F_2 generation, all with five toes symmetrically placed.

89 chickens were obtained, with the following digital classification:

89	{	Five-toe symmetrical	...	62
		Four-toe	„	7
		Six-toe	„	5
		a-symmetrical	15
				<hr/>
				89

The digital conformation of the a-symmetrical birds was as follows:

15	{	4 right, 5 left	4
		5 „ 4 „	2
		5 „ 6 „	5
		6 „ 5 „	3
		Abnormal second toe	...		1
					<hr/> 15

The following points deserve notice:

- (1) the high proportion of five-toed symmetrical birds, 62 out of 89;
- (2) the small number of four-toed symmetrical birds, 7;
- (3) the large number of a-symmetrical birds, 15 out of 89; among these latter the higher incidence of the extra digit (fifth or sixth toe) on the left side, is in harmony with previous observations, being in the proportion of nine on the left to five on the right;
- (4) the appearance of 13 birds having more than five digits on the right, or the left, or on both feet.

Thus, five birds had six digits on both feet; five had six digits on the right and five on the left foot; three only had six digits on the right and five on the left foot.

The occurrence of 13 birds with as many as six digits either symmetrically or a-symmetrically placed, calls for comment, especially as it may throw light on the nature of the genetic factor, which influences, or is associated with, the appearance of supernumerary digits.

The appearance of extra digits reached a maximum in one of these six-toed birds from this cross, a pullet, with Andalusian colouring, and the Houdan top-knot.

An X-ray photo of this bird shows the presence of an extra-metatarsal bone on both sides. In the left leg this bone enters above into the tibio-tarsal articulation on the inner or medium side, and after crossing obliquely behind the fused metatarsals which carry the three front toes, it ends below on the posterior aspect of that bone in two articular surfaces which carry two digits, one of which again divides and terminates in two toes, while the other ends in a single digit. In the right leg this extra metatarsal bone occupies the same position in the foot and ends below in the same way in two articular surfaces, one of which carries a single digit, and the other a proximal phalanx, which again ends in a double articular surface carrying two "image toes" (see illustrations, Figs. 1, 2, 3).

THE INFLUENCE OF A SMALLER DOSE ON THE SUPERNUMERARY
DIGIT FACTOR.

There is some evidence that a smaller dose of the supernumerary digit factor (whatever it be) derived from the Houdan or five-toed breed may influence the development of the terminal phalanx or phalanges of the normally placed hallux, without extending to the production of a second hallux above the normal one. The photographs on Pl. XX illustrate this point. The hind toe in a chicken of the Houdan-Andalusian cross, F_2 , shows on the left side a stunted, clawless hallux, while on the right side this digit is represented by a claw on one side of the digit. The abnormality also extends to the innermost of the three forward-pointing toes. This is short and stumpy, with a claw springing from the middle; the middle toe of the three is abnormally long (Fig. 4).

In two other birds from the same Houdan-Andalusian six-toed cock, but mated to Andalusian and Rhode Island cross F_1 hens (both four-toed breeds), the supernumerary digit influence in this diluted form resulted not in an extra digit above the normal hallux, but in an abnormal condition of the normally-placed hallux (Fig. 5).

In another case, also an a-symmetrical bird, the hallux on the left side was stumpy in shape and ended in two claws.

In another, also a-symmetrical, the hallux on the right side showed a similar change.

Thus, while the mating of the Houdan-Andalusian six-toed cock, with the Houdan-Andalusian F_2 hens, resulted in a large proportion of five-toed, a very small proportion of four-toed, and a considerable number of six-toed symmetrical, and five and six-toed a-symmetrical birds—the result of mating the same cock with Andalusian-Rhode Island hens (both four-toed breeds)—was a large preponderance of four-toed, a few five-toed, no six-toed and two a-symmetrical birds. In these latter the supernumerary digit influence showed itself in a duplication of the claws of the normally placed hallux (Fig. 5).

The question now arises, what light, if any, the appearance of six toes in a number of birds from this Houdan-Andalusian cross, when carried through three generations, throws on the nature of the genetic factor concerned in polydactyly in birds.

A sixth toe has occasionally been recorded in the Dorking breed (see Bateson, "Materials for the study of Variation"). In the present instance the sixth digit appeared in quite a number of birds, as the result of selective mating between a four-toed (Andalusian) and a five-toed (Houdan) breed.

The well-known proclivity of the Andalusian breed to segregate out as regards colour in the F_2 generation may have something to do with this result.

It is, I think, clear that the genetic factor, whatever its nature may be, which controls the development of the extra or fifth digit in the five-toed breeds, is not limited to that special digit, but influences or may influence all the digits, including the hallux on the inner or median aspect of the foot. It may even extend to the middle digit of the three forward toes, though in no case up to the present has the outer toe been abnormal. It may also bring about the appearance of an extra metatarsal bone. The conditions necessary to bring about this instability of meristic development seem to arise when the supernumerary digit factor is introduced by crossing into the genetic composition of a four-toed breed.

Whether the result of such mating in the zygotes of the F_2 and F_3 generations will be merely a terminal dichotomy, or a duplication, or a triplication of the normal hallux, or whether it will result in some abnormality of the inner or middle forward-pointing digits, or, as in an extreme case (see Fig. 3) in the appearance of an extra metatarsal bone, seems to depend partly on the dose of the supernumerary factor which has been introduced, and partly also on the particular breeds (four-toed and five-toed) used in the experimental crossing.

It would be useful to test all the known five-toed and four-toed breeds from this point of view.

I hope also to carry on the selective mating with the six-toed birds, both symmetrical and a-symmetrical, in order to test the possibility of establishing a homozygous six-toed breed.

DESCRIPTION OF PLATES XIX, XX.

PLATE XIX.

Fig. 1. Pullet. Houdan \times Andalusian cross, F_3 .

Fig. 2. Pullet. Houdan \times Andalusian cross, F_3 . Six digits on both feet. In the left foot the sixth toe passes down behind the partly webbed pair and the tip of the claw appears in the interspace between the hallux and the inner forward-pointing toe below.

Fig. 3. Pullet. Houdan \times Andalusian cross, F_3 . X-ray photo. The first phalanx and part of the termination of the sixth toe in the left foot can be made out in the interspace passing down behind the forward-pointing toes.

PLATE XX.

Fig. 4. Chicken. Houdan Andalusian cross, F_2 . Abnormal hallux on left foot. Abnormal hallux and inner and middle digits on right foot.

Fig. 5. Andalusian Rhode Island cross, $F_1 \times$ Houdan Andalusian cock. Dichotomy (terminal) of hallux on right side.

Fig. 6. Chicken from Houdan Andalusian cross, F_3 . Abnormal length and terminal duplication of extra hallux.

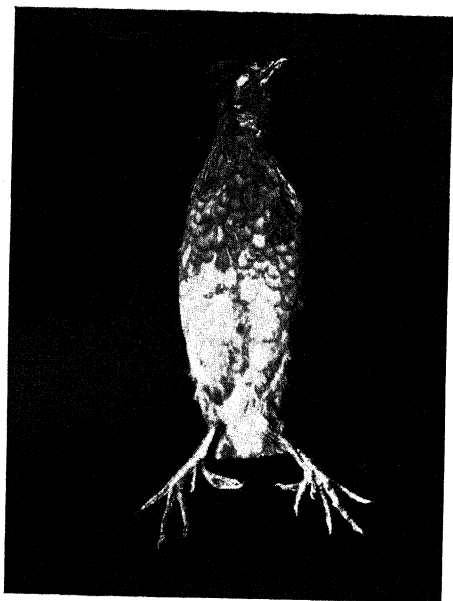


Fig. 1.



Fig. 2.

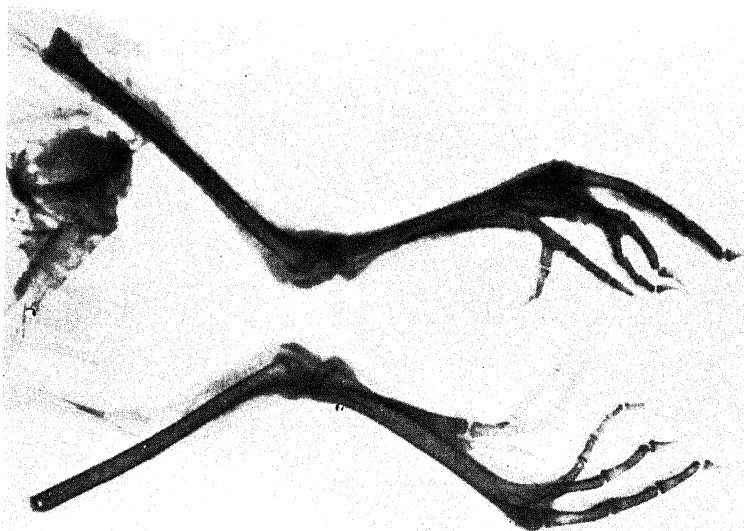


Fig. 3.

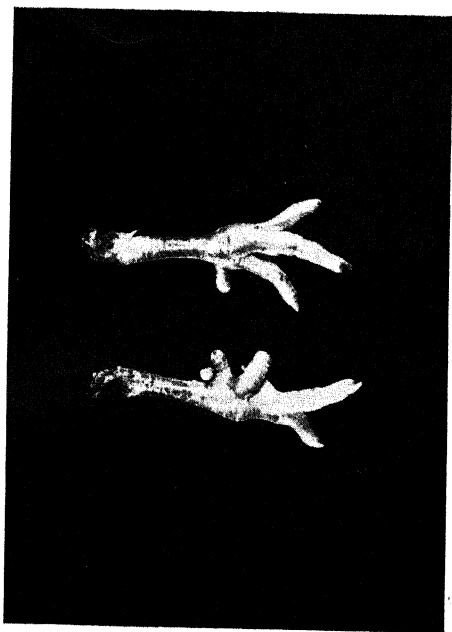


Fig. 4.

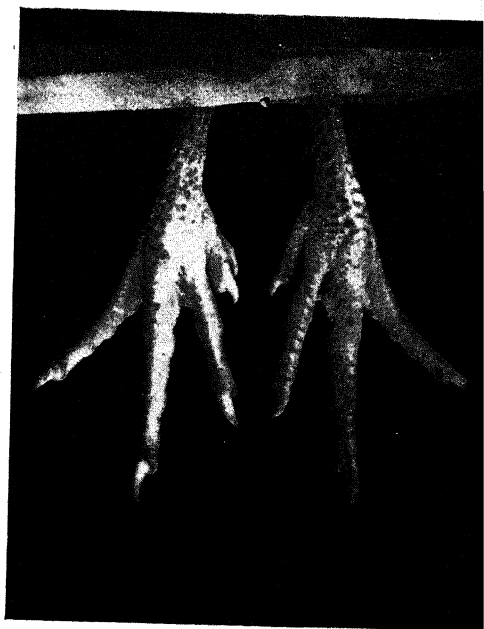


Fig. 5.

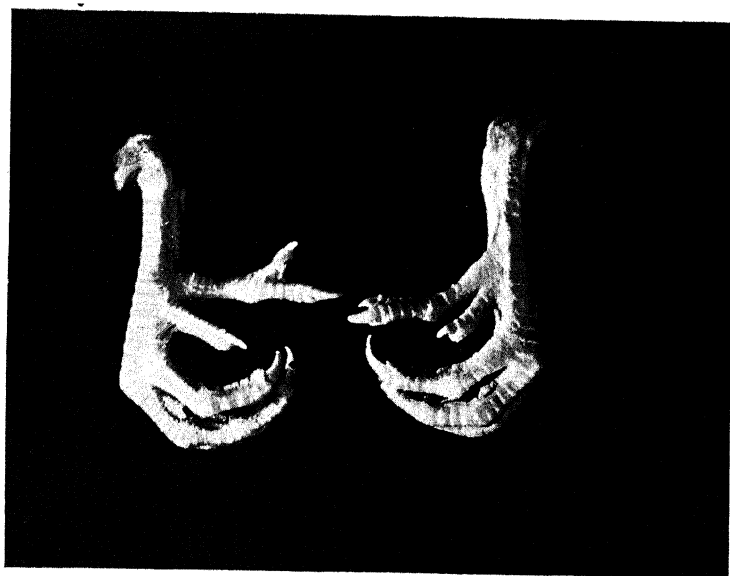


Fig. 6.



ON THE VARIATION AND INHERITANCE OF SOME MORPHOLOGICAL CHARACTERS IN *OPHRYOSCOLEX PURKYNJEI* (INFUSORIA OLIGOTRICHÁ).

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(With Two Text-figures.)

THE investigations of Jennings (1916) and his pupils (Hegner, 1918, 1919, Middleton, 1915), as well as those of Jollos (1921, 1924), established the presence of many different stocks, pure lines or families in several species of Protozoa. These stocks may differ in their physiological functions (rate of fission, endurance and so on), or they are distinguished by morphological features. Examples of the former are found in Infusoria and of the latter in Rhizopoda. It must be noted that the characters concerned in Rhizopoda (*Diffugia*, *Arcella*) relate to the form and size of the shell, or to the size and number of different shell-outgrowths (spines, teeth). The mode of inheritance of these characters during uniparental reproduction was also established by the authors mentioned above. It was shown that for instance the number of spines may vary between the parent and its direct offspring. A doubt may arise however whether the characters studied belong to the shell and not to the protoplasmic body of the animal, being that is to say alloplasmatic structures. Although we do not think this objection serious, still it seems well worth strengthening the results of previous authors by investigations on truly protoplasmic morphological characters in Protozoa.

Some of the Ophryoscolecidae infesting in great numbers the stomach of oxen, sheep and other Ruminantia seem to be well adapted for such studies. They possess many very strongly developed morphological characters (for instance the number, size and mode of branching of caudal spines), facilitating the study of variation and inheritance. A form especially suited for such work is *Ophryoscolex purkynjei* with several circles of spines surrounding the posterior end of the body. These spines do not represent simple cuticular outgrowths but are of a very complex structure, consisting of ectoplasm with interior skeletal rods and contractile fibrils, the whole covered with a cuticular sheath. Therefore in

examining the variation and inheritance of these spines we have to do with a typical protoplasmic part of the body.

Two questions are dealt with in this paper: (1) can the species *O. purkynjei* be divided into several stocks differing in their morphological characters? as such characters we consider the number of branches in the forked spines of the first circle; (2) is the character of branching of spines retained or changed in daughter-individuals produced by fission from the same parent?

The most important points in the morphology of Ophryoscolex purkynjei.

This infusorian has a somewhat barrel-like body, 150–200 μ long and 80–100 μ wide. The anterior end of the body is occupied by a wide peristome, surrounded by a spirally disposed adoral zone of membranelles. Another set of membranelles (the dorsal zone) is situated on the dorsal side of the animal. This zone encircles not only the back but also the lateral sides of the body. It forms a sort of crescent, the membranelles being absent on the ventral side of the body only. The dorsal zone divides the body into two parts: anterior or prezoal, and posterior or postzoal. The prezoal part has the appearance of a truncated cone with a completely smooth surface. The postzoal part possesses on its dorsal and lateral sides seven shallow longitudinal grooves, as in the surface of a peeled orange. The ventral side of the postzoal part remains grooveless. These grooves divide the postzoal part of the body into seven longitudinal sections: the largest of them occupies the ventral side of the animal, while the remaining six (all of them nearly of the same width) take up the lateral sides of the infusorian and its back. We designate the six latero-dorsal sections as sections I–VI, numbering from left to right. Sections I–V contain the contractile vacuoles (nine in number); section VI has no vacuoles because it is occupied by a sausage-shaped macronucleus, underlying the cuticle.

The posterior end of the body is elaborately adorned with numerous spines whose distribution is very constant. The ventral section of the postzoal part gradually tapers backwards until it becomes a strong ventral spine protruding at the posterior end of the animal. The right side of the ventral spine is furnished at its base with a secondary spine. The remaining sections of the body bud off three circles of lesser spines. The first or anterior circle is formed by six large spines, bi-, tri- or quadri-furcated at their free end. All the branches or teeth of a spine lie in one plane, the spine having a flattened aspect. In very rare cases (three times in several hundred animals investigated) the number of

branches may rise to five. There may be differences in the mode of branching of the spines. In most cases all the branches of a spine are equally well developed. In other and rarer cases there are two or three equal branches, one of which is split in two or gives off a collateral branch, by which process the whole number of branches is increased to three or four. Every spine exhibits a very complex structure. It consists of an ectoplasmic outgrowth covered with a thin cuticle and supported

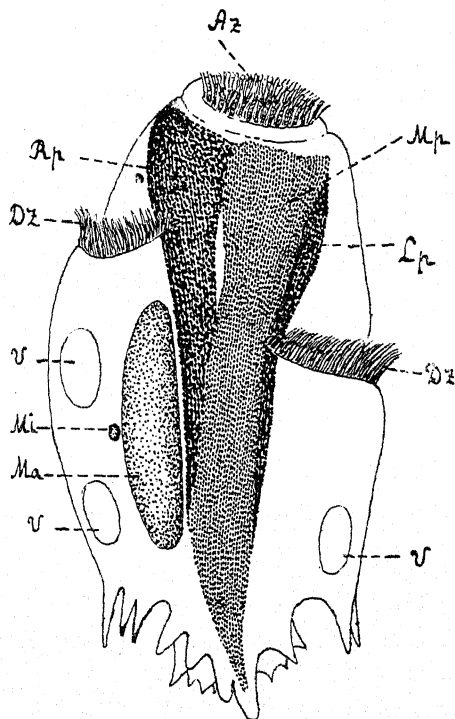


Fig. 1. *Ophryoscolex purkynjei* seen from the ventral side. *Az*, adoral zone of membranelles; *Dz*, dorsal zone of membranelles; *Lp*, *Mp*, *Rp*, left, ventral and right skeletal plates; *v*, contractile vacuoles; *Ma*, macronucleus; *Mi*, micronucleus.

by an inner skeletal frame. This latter has the form of a semicircular arc, occupying the base of the spine, from which diverge 2-4 thin skeletal rods entering the branches of the spine and penetrating to their free end. Moreover the ectoplasm of the spines contains a system of thin muscular fibrils or myonemata.

The second circle of spines is situated behind the first one and consists of about twenty (this number can vary) independent short spines

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projecting from the border of a circular rim which surrounds the posterior end of the body. The third circle contains only 5-7 spines lying at the base of the large ventral spine just inside the second circle.

The present paper deals exclusively with the variation and inheritance of the number of branches in the spines of the first circle.

At the bottom of the peristome lies the mouth which opens into a strongly developed pharynx. The latter extends to the hinder part of the body, being closely applied to the right surface of the body. Between the pharynx and the body-wall are inserted three skeletal plates which support the right, the left and the ventral sides of the gullet. These plates consist of a substance nearly akin to the cellulose of plants and are vividly stained a dark violet by chloro-iodide of zinc, and blue by iodine and sulphuric acid. The right and the ventral plates fuse together and continue far into the base of the big ventral spine. For present purposes there is no need to describe further cytological details of *Ophryoscolex purkynjei*.

Variation of the number of branches in the spines of O. purkynjei.

On examining different specimens of the same population of *O. purkynjei* it is easy to notice that the number of branches in the spines of the first circle can vary to a great extent. The fundamental form (and the only one known heretofore from the descriptions of Eberlein and others) of the spines appears to be a three-branched one. This ground-plan is in many specimens retained by the majority of the spines. Sometimes (but not very often) all the spines of a specimen are three-branched. Yet usually some of the spines exhibit a plus- or minus-variation, producing two, four or (very seldom) even five branches. In different specimens of the same population the number and the position of the two- or four-branched spines may vary. So for instance we have the following differences in 10 specimens taken at random from the population No. 248.

Number of branches in	Specimen									
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10
Spine No. I	4	3	4	4	3	4	3	4	3	3
Spine No. II	4	2	3	3	3	3	3	3	2	2
Spine No. III	3	2	2	3	2	3	2	3	2	2
Spine No. IV	3	3	3	3	3	3	3	3	2	3
Spine No. V	3	2	3	3	3	3	3	3	4	3
Spine No. VI	3	3	3	3	3	3	3	3	3	3

Still there always exists in the same population a tendency in some spines to augment and in others to reduce the number of their branches. So for instance in the population No. 43 the second spine in 36 cases out

of 50 has only two branches, while the first spine possesses two branches only in 2 specimens out of 50. Or, to take another case, in the population No. 248 the first spine possesses four branches in 22 specimens out of 50, while the spines III and IV exhibit the same feature only in 1 specimen out of 50.

The study of populations taken from different specimens of the host has shown that they differ in their mode of variation, each population possessing its own characteristic features. It can be best exemplified by a comparison of several populations tabulated below. Table I is prepared after an examination of six sets of 50 specimens each, taken from different populations (Nos. 43, 242, 248, 250, 254 and 260).

TABLE I.

Showing the character of branching in six different populations of O. purkynjei. For every population are examined 50 specimens taken at random.

	Absolute number of spines						The same in percentages					
	Population No. 43	Population No. 250	Population No. 260	Population No. 254	Population No. 248	Population No. 242	Population No. 43	Population No. 250	Population No. 260	Population No. 254	Population No. 248	Population No. 242
Spine I												
Bifurcated	1	2	1	—	—	—	2	4	2	—	—	—
Trifurcated	46	35	38	35	28	31	92	70	76	70	56	62
Quadrifurcated	3	13	11	15	22	19	6	26	22	30	44	38
Spine II												
Bifurcated	36	27	4	4	14	2	72	54	8	8	28	4
Trifurcated	14	20	42	38	33	42	28	40	84	76	66	84
Quadrifurcated	—	3	4	8	3	6	—	6	8	16	6	12
Spine III												
Bifurcated	39	32	12	13	14	7	78	64	24	26	28	14
Trifurcated	11	17	35	36	35	39	22	34	70	72	70	78
Quadrifurcated	—	1	3	1	1	4	—	2	6	2	2	8
Spine IV												
Bifurcated	35	17	10	11	5	6	70	34	20	22	10	12
Trifurcated	15	33	36	34	44	43	30	66	72	68	88	86
Quadrifurcated	—	—	4	5	1	1	—	—	8	10	2	2
Spine V												
Bifurcated	22	8	9	5	1	3	44	16	18	10	2	6
Trifurcated	27	41	39	39	43	36	54	82	78	78	86	72
Quadrifurcated	1	1	2	6	6	11	2	2	4	12	12	22
Spine VI												
Bifurcated	13	6	4	6	1	1	26	12	8	12	2	2
Trifurcated	34	38	45	41	40	37	68	76	90	82	80	74
Quadrifurcated	3	6	1	3	9	12	6	12	2	6	18	24
All the spines												
Bifurcated	147	92	40	39	35	19	49	31	14	13	12	6
Trifurcated	146	184	235	223	223	223	49	61	78	74	74	76
Quadrifurcated	7	24	25	38	42	53	2	8	8	13	14	18
Total of spines	300	300	300	300	300	300	100	100	100	100	100	100
Total of branches	756	832	885	899	907	934						

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It is clear that these six populations can be ranged in two groups. One of the groups is characterized by a tendency to a minus-variation. Indeed in the populations Nos. 250 and 43 a great number (from 31 per cent. to 49 per cent.) of spines are bifurcated. Another group has no such marked tendency to bifurcation, but on the contrary may exhibit a large number of quadrifurcated spines. So in the populations 242, 248, 254 and 260 there are only 6-14 per cent. of bifurcated spines, while 8-18 per cent. of the spines become quadrifurcated, whereas populations 250 and 43 exhibit respectively 8 and 2.5 per cent. of quadrifurcated spines.

These facts seem to show definitely: (1) that there exist many races or pure strains of *O. purkynjei* differing in morphological characters; (2) that the characters named vary to a certain degree in the members of the same population.

Some of the spines have in all the populations an unquestionably stronger propensity to a plus- or minus-variation than the others. So for instance spine I has a marked tendency to increase the number of its branches; even in populations characterized by a preponderance of minus-variations in other spines spine I has more branches than the remaining ones. In the six thoroughly studied populations spine I was quadrifurcated in 6 per cent. (population No. 43), 22 per cent. (No. 260), 26 per cent. (No. 250), 30 per cent. (No. 254), 38 per cent. (No. 242) and 44 per cent. (No. 248) of all the specimens. Meanwhile a bifurcated spine I was found in three of the populations only, and then only in 2 per cent. (Nos. 43 and 260) to 4 per cent. (No. 250) of all the specimens examined. Or if we put together the results for all the populations the mean percentage of quadrifurcated first spines is 28 per cent., while that of bifurcated is 1.3 per cent. only.

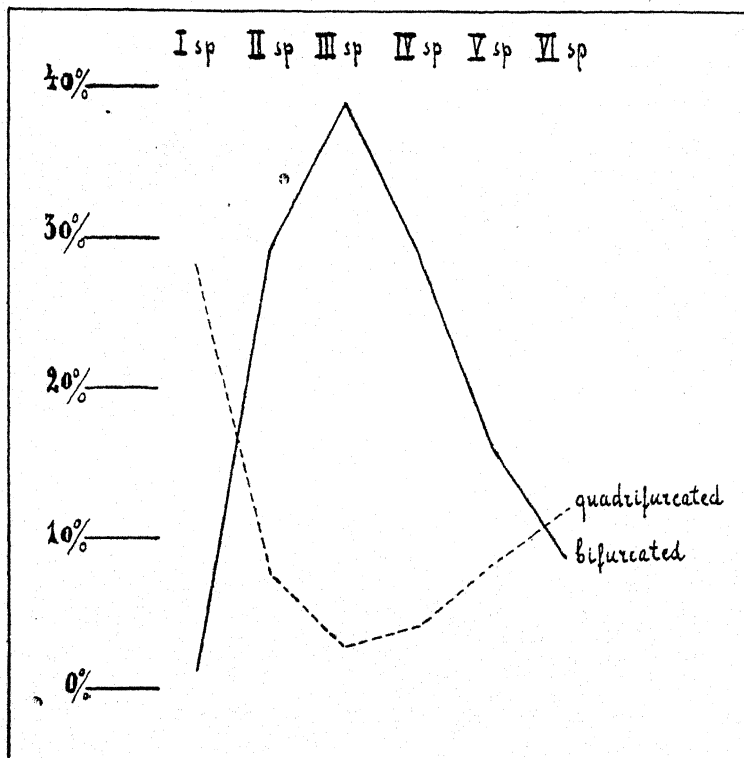
Let us take for comparison spine III which has the tendency to form minus-variations. It is quadrifurcated in 2-8 per cent. of all the cases; bifurcated III spines are found in 14 per cent. (population No. 242), 24 per cent. (No. 260), 26 per cent. (No. 254), 28 per cent. (No. 248), 64 per cent. (No. 250) and 78 per cent. (No. 43) of the whole population. The mean for six populations is: 3.3 per cent. of quadrifurcated and 39 per cent. of bifurcated III spines.

Comparing the data from 11 different populations of *O. purkynjei* for different spines of the first circle we remark that the disposition to minus-variations is most strongly developed in spine III (39 per cent. of bifurcated spines); from here this tendency decreases in both directions, i.e. towards spine I as well as towards spine VI (see Table II).

Plus-variations are most frequently met with in spine I; then their number diminishes in spine II and reaches its minimum in spine III; from here on the number of quadrifurcated spines again increases towards spine VI.

There is a reason why spine III exhibits such a slight tendency to increase the number of its branches. As we shall show in our

TABLE II.



detailed monograph of the *Ophryoscolecidae*, the nearest but more primitive allies of *O. purkynjei* possess only five bifurcated spines (I-V). Spine VI is a character newly acquired in this species, as well as in *O. caudatus*. Now in the above-mentioned more primitive species of *Ophryoscolex* (for instance in *O. diplospinosus* Dog. and Fed.) spine III corresponds to the median dorsal line of the body, lying just opposite to the big ventral spine. The latter (as we have seen in *O. purkynjei*) remains always unbranched, which character is also for a

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long period preserved by its dorsal partner. On both sides of the dorso-ventral plane the tendency to form branches gradually increases in the ventral direction reaching its height in spine I and in (the newly acquired) spine VI.

The differences in the mode of branching in different populations are well shown if we try to compare the whole number of branches existing on the spines of the same number of specimens taken from different populations. Let us take lots of 50 individuals each from the six populations already mentioned above. Each lot possesses $50 \times 6 = 300$ spines. These 300 spines have on the whole in different populations the following number of branches:

Population No. 43—756 branches.	The average number of branches on a spine is 2.50				
No. 250—832	"	"	"	"	2.77
No. 260—885	"	"	"	"	2.95
No. 254—899	"	"	"	"	3.00
No. 248—907	"	"	"	"	3.00
No. 242—934	"	"	"	"	3.11

It is easy to notice that the difference between two populations (for instance Nos. 43 and 242) can be very considerable.

Inheritance of the number of branches in the spines of O. purkynjei during the asexual reproduction.

The reproduction is effected by a process of binary fission. The animal becomes constricted transversely on the level between the two rows of contractile vacuoles and gradually divides into an anterior and a posterior daughter-individual (Fig. 2). The posterior one gets the spine-armature of the parent, while the anterior forms the spines anew.

The branching of the new spines of the first circle begins before the separation of the daughter-individuals so that it is possible to compare the character of spines and their mode of branching in both the individuals. In this way one can judge if the character of branching in the parent is retained by the anterior daughter-individual. It is difficult to get numerous specimens in the latest stages of fission, but still we succeeded in collecting three lots of such individuals from different populations (Nos. 250, 254 and 260). One of the lots (No. 260) contained 20 specimens, while the other two (Nos. 250 and 254) were of 15 specimens each.

The first result of the investigation was that the newly arising spine of the anterior daughter individual may often differ in the number of

its branches from the corresponding old spine of the posterior individual. This difference can manifest itself in an increase as well as in a decrease in the number of branches in the new spine. One and the same individual can exhibit in different spines both these processes.

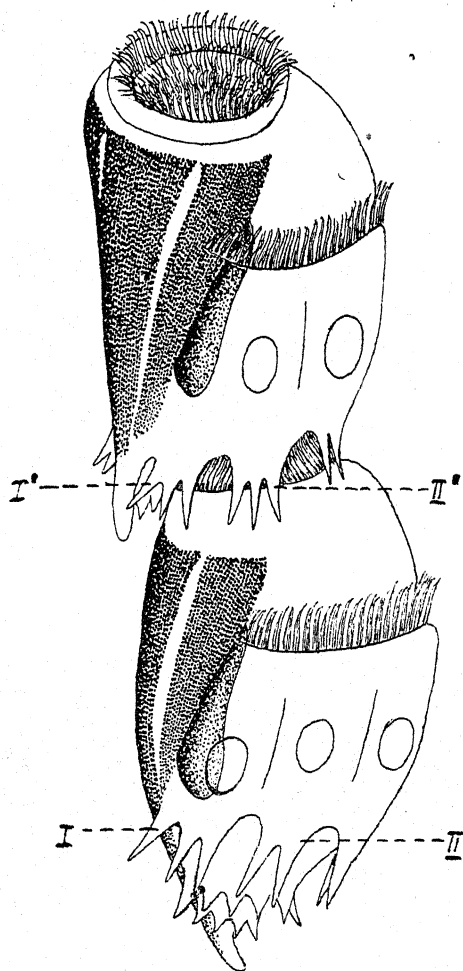


Fig. 2. *Ophryoscolex purkynjei* in a late stage of fission; I and II, first and second branched spines of the posterior daughter-individual; I' and II', first and second branched spines of the anterior individual.

So for instance specimen No. 14 from the population 250 had the following spine-formula for the posterior (below the horizontal line) and anterior (above the horizontal line) daughter-individual:

€	Spines					
	I	II	III	IV	V	VI
	3	2	3	4	3	3
	3	3	3	3	3	3

Such instances are by no means rare. On the contrary the number of specimens where the character of branching in both daughter-individuals completely coincides is very small. Out of the whole number of 50 specimens examined there were seven such fission-stages (or about 15 per cent.). Very often (see Table III) the anterior individual of an *Ophryoscolex* in a state of fission differs from the posterior one in the mode of branching of one of the spines only. Less often the difference was observed in two, three, or once even in as many as four of the spines.

TABLE III.

To show the inheritance of the branching of spines in the dividing specimens of the lot No. 254.

The lot contains 15 specimens.

		Spines								Spines					
		I	I	III	IV	V	VI			I	II	III	IV	V	VI
No. 1	Anterior	4	3	3	3	3	3	No. 9	Anterior	4	3	3	3	3	3
	Posterior	4	3	3	2	3	3		Posterior	3	3	3	3	3	3
No. 2	Anterior	3	3	3	3	4	3	No. 10	Anterior	3	3	3	3	3	3
	Posterior	3	3	2	3	4	3		Posterior	4	3	3	4	4	3
No. 3	Anterior	3	3	3	3	3	3	No. 11	Anterior	3	4	4	3	3	3
	Posterior	4	3	3	3	3	3		Posterior	3	3	3	3	3	3
No. 4	Anterior	3	3	2	3	3	3	No. 12	Anterior	3	3	3	3	3	2
	Posterior	4	3	3	4	3	3		Posterior	4	4	3	3	3	2
No. 5	Anterior	3	3	3	3	3	2	No. 13	Anterior	3	3	3	3	4	2
	Posterior	3	3	3	3	3	3		Posterior	4	4	3	3	4	3
No. 6	Anterior	3	3	3	2	3	3	No. 14	Anterior	3	3	3	2	3	3
	Posterior	3	3	3	3	2	3		Posterior	4	3	3	3	3	3
No. 7	Anterior	3	3	2	3	3	2	No. 15	Anterior	3	3	2	3	3	3
	Posterior	3	3	3	3	3	2		Posterior	3	3	3	2	4	3
No. 8	Anterior	4	3	3	3	3	3								
	Posterior	3	3	3	3	3	3								

For the 20 cases from population No. 260 the total number of spines dealt with belonging to the anterior individuals was $6 \times 20 = 120$, and for the 15 cases from populations No. 250 and 254 respectively was $6 \times 15 = 90$. Amongst these there were in the lot No. 260, 26 spines (or about 21.6 per cent.) differing from the spines of the posterior individual. In the lot No. 250 there were 24 such spines (or 26 per cent.), while the lot No. 254 possessed 27 such modified spines (or 30 per cent. of the whole number). Therefore at every fission the number of branches

is changed in about 20-30 per cent. of spines of the anterior daughter-individuals.

Is the disposition to vary expressed in all the spines in the same degree? The number of the specimens investigated is too small to permit us to solve this question. Still if we may judge from our scanty material the most variable of the spines are III and IV. In these spines the

TABLE IV.

	15 specimens of the Population No. 250		15 specimens of the Population No. 254		20 specimens of the Population No. 260	
	Posterior daughter- individ.	Anterior daughter- individ.	Posterior daughter- individ.	Anterior daughter- individ.	Posterior daughter- individ.	Anterior daughter- individ.
Spine I						
Bifurcated	—	—	—	—	1	1
Trifurcated	9	10	12	8	14	17
Quadrifurcated	6	5	3	7	5	2
Spine II						
Bifurcated	5	7	—	—	—	1
Trifurcated	9	7	14	13	20	18
Quadrifurcated	1	1	1	2	—	1
Spine III						
Bifurcated	5	7	3	1	3	4
Trifurcated	9	8	11	14	16	16
Quadrifurcated	1	—	1	—	1	—
Spine IV						
Bifurcated	2	4	2	2	1	4
Trifurcated	11	11	13	11	18	13
Quadrifurcated	2	—	—	2	1	3
Spine V						
Bifurcated	2	—	—	1	3	3
Trifurcated	12	14	13	10	16	16
Quadrifurcated	1	1	2	4	1	1
Spine VI						
Bifurcated	—	1	4	2	2	3
Trifurcated	13	13	11	13	17	16
Quadrifurcated	2	1	—	—	1	1
All the spines together						
Bifurcated	14	19	9	6	10	16
Trifurcated	63	63	75	69	101	96
Quadrifurcated	13	8	6	15	9	8
Total of spines	90	90	90	90	120	120
Total of branches	269	259*	267	279	359	352

number of branches was changed in 34 and 36 per cent. respectively of the anterior daughter-individuals. Spine VI proved to be the most constant of the spines, as it altered the number of its branches in 14 per cent. of the individuals only. It is noteworthy that the newly acquired spine VI is less variable than the rest of the spines which are of more ancient origin.

Our data are too few to enable us to elucidate the question whether

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the general character of a population is on the whole maintained throughout the life of the said population in the same host; or whether the character of branching may change in the course of generations arising by asexual reproduction. In the three lots examined the number of trifurcated spines was slightly increased in the anterior individuals in comparison with the posterior ones.

The high degree of variation in the number of spine-branches must perhaps be attributed to the following circumstance. The comparison of *O. purkynjei* with several other species closely related to it proves that *O. purkynjei* represents a species which has not yet attained the state of stable equilibrium. This species is on the way to increase the number of the branches in the spines of the first circle from two to four.

SUMMARY.

1. The branched spines of *O. purkynjei* represent very complicated protoplasmic structures.

2. The number of branches in the spines of *O. purkynjei* varies from 2-4 in different members of the same population and in different spines of the same individual.

3. Every population possesses a somewhat different character of branching, which circumstance proves the existence of several different strains or races in the species named.

4. Spine I possesses in the highest degree a tendency to form plus-variations, while spine III is characterized by its disposition to minus-variations. This character remains true for all the populations examined.

5. The examination of 50 individuals in the late stages of fission proves that the newly arising spines of anterior daughter-individuals may very often differ in the number of branches from the corresponding old spines of posterior daughter-individuals. This difference manifests itself in an increased, as well as in a decreased, number of branches in the new spines.

AN EXPERIMENTAL STUDY ON XENIA IN THE DOMESTIC FOWL.

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(With Three Text-figures.)

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INTRODUCTION.

WE call animal xenia the change of colour of birds' eggs, which is said to take place when mating females with males of a breed characterised by eggs of a different tint. We are therefore dealing here with direct changes of egg-colour of the females examined, i.e. of the P generation, and not with the colour of eggs from F_1 hens. Such changes have been mentioned by v. Nathusius (11, 12), Kutter (9) and Seidlitz (15), who noticed that hens with white eggs began to lay more or less yellowish eggs a few days after having been mated with a Cochin cock. An analogous phenomenon, although in an opposite direction, was later described by Holdefeiss (5), who observed that the eggs of Plymouth Rocks (i.e. of a breed having brown eggs) become lighter in tint when the hens are mated with a cock of Italian breed characterised by white eggs. The best known are undoubtedly the researches undertaken in this direction by A. v. Tschermak. In his first papers (16, 17) this physiologist emphasises the changes which he states that he noticed in the eggs of *Fringilla canaria* mated with *F. spinus*, *F. cannabina*, *F. serinus*, *F. carduelis* and *Pyrrhula rubicilla*; in the last (18) he describes the phenomenon of xenia in several crosses of fowls. An analogous phenomenon in fowls has also been noticed, at least in one case, by

Walther(20). Goldschmidt(3) observed that a duck of Cayuga breed with dark eggs crossed with a drake of Pekin breed (having white eggs) laid white eggs. This author does not however attribute any essential importance to this case, as the duck examined was genetically of a mixed character, and he recommends a continual control of the material. I also observed occasionally during my experiments on the inheritance of egg-colour in fowls that the Polish Greenlegs mated with a cock of the same breed laid cream eggs characteristic of this variety, whereas the next year, after having been crossed with a Leghorn cock, they produced distinctly lighter eggs. I supposed that this phenomenon may possibly be brought about by xenia, but I emphasised that, owing to lack of exact knowledge of the natural, periodical variability of egg-colour in fowls, all that has been described in this direction "only points very distinctly to the phenomenon of xenia of eggs *without proving it methodically*" (Kopeć(6), p. 338).

In respect to natural colour variability of birds' eggs it ought to be emphasised that v. Tschermak (18), p. 51) appears to take it into consideration. As an instance of such changes the author describes a lightening of the eggs laid by hybrids of Minorca hen mated with a Cochin cock, where "die Eifarbe blasste binnen 1½ Monaten in der Beobachtungszeit vom 5. ii. bis 25. iv. 1914 von dem ursprünglichen hellgelbbraun, allmählich fortschreitend, ab bis zu einem weiterhin recht stabil bleibenden schwach bräunlichem Weiss." v. Tschermak believes, it is true, that a "hyperkritischer Beurteiler" may reduce the phenomena he relates as xenia to such "spontan erfolgende 'Altersveränderung' der Pigmentierung," and in spite of the above observations he categorically opposes such an interpretation. It would be, according to his opinion, astonishing if "eine spontane Altersveränderung bei den weisseiigen Rassen in einer Zunahme der Pigmentierung, bei den brauneigen Rassen gerade umgekehrt in einer Abnahme der Pigmentierung gelegen wäre." In respect of this argument of v. Tschermak the following remark should be made. A xenial darkening of the eggs was obtained by this author in three crosses, viz. ♀ White Italian × ♂ Langshan, ♀ Partridge Italian × ♂ Plymouth Rock and ♀ White Minorca × ♂ Cochin, and an analogous lightening in the three reciprocal crosses, but only *one* hen in each experiment was used¹. Each hen was kept alter-

¹ v. Tschermak's paper is considered by him as a report of the first series of experiments, to be succeeded by further researches on a reduced number of breeds and an increased number of specimens. So far as it is known to me the results of these investigations have not hitherto been published.

nately with a cock of the same breed (periods of pure culture) or with a cock of a different breed (periods of hybridisation). As the material of v. Tschermak consisted of only six experimental specimens, no control material being taken into consideration, the author can compare the eggs from the period of hybridisation with those of the same hen from the preceding period of pure culture, but he is unable to say how eggs from fowls which have never in their life been mated with a cock of a foreign breed with differently tinted eggs would behave during the same life or year period. On this account the increase of the range of variability of egg-colour during the periods of hybridisation is not a valid argument. Consequently v. Tschermak's opinion, that it would be strange if directly opposite changes in white and brown eggs were observed during one and the same period of hybridisation, is seemingly true, but requires an impartial confirmation on adequate control material of eggs from the breeds concerned. As v. Tschermak had no such control birds throughout the whole time of his experiments to give him standard eggs for comparison during every period of the life of the experimental hens, the problem of a specific "telegonical" character of certain other changes described by this author remains, according to my opinion, not satisfactorily solved (cf. also the general discussion below).

Moreover I believe that the experiments hitherto made on xenia in fowls can hardly be considered as conclusive on account of further methodical inaccuracies. First of all, as to the number of eggs disposed of by separate investigators, we are either not informed at all (v. Tschermak(18)), or we see that the material was scanty, as in the research of Holdefleiss(5) where the number of eggs from the two hens examined was only 92. Walther's(20) observations are based, it is true, on 630 eggs from 13 hens, but he introduces as many as six breeds, therefore considerably reducing the number of hens and eggs in each cross.

The method of estimation of the egg-colours by mere inspection, adopted by separate authors, in spite of a considerable range of variability of egg-tint emphasised by them in certain cases, does not seem to me to allow critical analysis of the observations. The authors either discriminate only a few colours, e.g. "weiss," "braun" and "mittel," or content themselves with a cursory report of a certain darkening or lightening of the hue. Especially in the experiments of Holdefleiss it ought to be remarked that from the 92 eggs examined by him only three chickens hatched at all, while in numerous other eggs no trace of development was observable. It is therefore doubtful whether the hens

he examined were permanently fecundated by a cock of a different breed, i.e. whether foreign sperm was really present in their sexual ducts. The deductions which may be drawn by a sceptical reader from material not scrutinised in this respect are well understood by Walther, who therefore first of all bases his research on the eggs from which chickens did hatch. v. Tschermak on the contrary does not seem to pay attention to this methodically important circumstance. And here it should be remarked that, as a rule, the above-mentioned authors have not recorded the colour of the eggs laid by the F_1 hens, although only by the examination of the egg-colour of this generation can we check the results obtained on xenia. I believe that, without ascertaining the intermediate character of the egg-colour in F_1 hens, the positive results on xenia may easily be reduced to secondary tint variations, having no connection with the phenomenon under examination; and *vice versa* no inferences ought to be drawn from negative results, if the colour of the eggs laid by F_1 hens exhibits complete dominance of either parental egg-hue, because it is obvious that in the latter case the experiments were made with unsuitable material¹.

As I have never bred canaries, I can discuss the papers of v. Tschermak (16, 17) on xenia in these birds only from a theoretical point of view. Normal, fecundated eggs of this form had "unscharfe, hellbraune, polygonale bis rundliche Flecken" on a bluish-green ground. On the other hand, the eggs of greenfinches, goldfinches and other wild forms which were used in crosses, showed, apart from analogous light brown spots, "eine ganz charakteristische schwarzbraune Zeichnung," i.e. dots, spots, commas and lines on a yellowish-white or somewhat greenish ground. On the eggs of canary-birds mated with the above-mentioned wild species the appearance of this black-brown marking (absent in normal canaries' eggs) was ascertained by v. Tschermak and considered as an unquestionable proof of appearance of xenia. v. Tschermak remarks, it is true, that the black photographs of his paper are not sufficient to produce conviction as to the truth of his observations, but I do not suppose that anybody would call in question these assertions. My criticisms do not refer to the facts, but to the decisive conclusions of v. Tschermak as to the indubitable character of xenia of this phenomenon. It is very characteristic that the tint of the ground,

¹ I am not able to report the methods of the earlier observations by v. Nathusius, Kutter and Seidlitz, as they were inaccessible to me. I do not believe, however, that v. Tschermak, who seems to know the original papers, would consciously degrade his methods as compared with the proceeding of his precursors.

supposedly the most liable to changes, remains unaltered in all crosses. On the other hand, it is striking that, as it is noticed by v. Tschermak in pure-bred canary-birds also, "ganz selten kommen völlig vereinzelte schwarzbraune Pünktchen auf der Stumpfpolhälfte vor" (v. Tschermak (17), p. 373). I do not know whether and to what degree variability of egg-colour throughout their whole life has been examined in canaries. From v. Tschermak's paper (17), p. 387 we learn however that, according to Paessler (13) and Wickmann (21), "die meisten Raubvögel, welche sonst weisse Eier legen, mitunter gefleckte produzieren und umgekehrt." (Cf. here also the great variability of egg-colour noticed in *Corvus corax* L. by Krause (10) and in *C. frugilegus* L. by Tur (19).) I want to remark, moreover, that the "xenial-eggs" of canary-birds were compared by v. Tschermak with eggs from greenfinches, goldfinches and other wild forms, derived, it is true, from known sources, but laid by apparently totally foreign females, not genetically connected with the males used in the crosses.

Consequently I do not consider the appearance of xenia either in fowls or in canary-birds as proved. From the fundamental research of Punnett and Bailey (14) on heredity of egg-colour in poultry it is clear that these authors did not succeed in observing such phenomena, and P. Hertwig (4) has also called into question the phenomenon of xenia in fowls.

MATERIAL AND METHODS.

My research on xenia in fowls was partly published elsewhere¹. The present paper contains more detailed materials and observations. The experiments were performed on two breeds: on two years old White Leghorns which lay as a rule chalk white eggs, and on Buff Orpingtons of the same age, with more or less brown eggs. A darkening of the eggs from Leghorn fowls kept with an Orpington cock, or a lightening of the eggs from Orpingtons bred with a Leghorn cock would prove the existence of xenia. Thus the investigations were restricted to a single reciprocal cross, and the basis of this one experiment could be quantitatively enlarged. In order to obtain a suitable standard material of eggs for comparison, some fowls of the same age, both Leghorns and Orpingtons, were bred throughout the whole time of the investigations separately without a cock. The eggs from these hens served as a permanent control of the natural periodical variability of egg-colour

¹ Cf. *Mém. de l'Institut Nat. Polonais d'Économie rurale à Pulawy*, VI, 1925, presented November 2nd, 1924.

in fowls. My object was to examine whether the phenomena of xenia noticed by the foregoing authors might not be reduced to purely physiological variability.

I attempted also to test the phenomenon of xenia by further direct experiments. For this purpose Leghorn hens were injected with testicles from Orpington cocks and Orpington hens with the sexual glands from Leghorn males. The injections were made six times, i.e. on May 12th, 17th and 26th and on June 4th, 12th and 19th, 1923. The cocks were quickly killed and the aseptically removed testicles were ground in a sterilised mortar with the addition of from 20 to 30 c.c. of physiological salt solution. The thick fluid obtained was introduced directly into the abdominal cavity of the hen by means of a Prawatz syringe with a blunt needle. Each Leghorn fowl was every time injected with 0.5, each Orpington with 0.4 of a foreign testicle. The fowls were carried over again to their hen-houses, no symptoms whatsoever of disease being observed. If the investigations which refer the shell pigments to the blood or to its decomposition products are right¹, it might have been supposed that in my experiments, where during five weeks as much as three complete Orpington testicles had been introduced directly into the abdominal cavity of each Leghorn fowl, and 2.4 Leghorn testicles into each Orpington hen, a much greater influence on egg-colour ought to be observable than during normal crosses with a properly chosen cock.

All the hens examined from either race were sisters derived from the same hatching in 1921. They began to lay in the spring of 1922. All fowls were from thorough-bred material kept by the author for several years. The specimens belonging to either breed and to each series of experiments were kept in separate hen-houses provided with running yards of the same dimensions, enclosed with wire nets. The food was the same for all hens during the whole time of observation. The eggs were checked in trap-nests. The egg production was in general scanty, undoubtedly partly owing to the small running yards. During the period from November 1st till January 1st in 1923 only the Orpington hens laid some eggs.

For the determination of egg-colour I used a graduated set of tints arranged *ad hoc* by means of thoroughly blown shells of all the 13 tints which have been discriminated, from the lightest hue of the Leghorn eggs to the darkest colour observed in Orpingtons. All the eggs which have been laid were examined, not one being rejected. Owing to changes of egg-colour which occur with time the tint of the egg was determined

¹ For references see the paper of Giersberg (2).

on the day on which the egg was laid. The shells which constituted the set of colour standards were kept in a dry and dark room. When the tint of any shell of this set underwent any change it was replaced by a fresh one.

The observations lasted from April 1st, 1923, till July 1st, 1925, and they were divided as a rule into periods of two months. The average egg-colour in degrees of the standard scale was determined for each period separately. As the changes of egg-colour, which might have been noticed during the observations, were identical in separate fowls of one material, the general average intensities were also denoted for all eggs of one period from all hens of one material together. In 1923 as well as in 1924 many eggs from hens kept with a cock of different breed were incubated during different seasons, and the eggs proved fertile. The plumage colour of the F_1 generation was more or less distinctly that of hybrids. Several hens, one year old, of this generation were examined in regard to the colour of their eggs. The hens Nos. 1, 20 and 33 died before the observations were finished. The total material consisted of 3577 eggs from 38 hens, viz. 487 eggs derived from five Leghorn hens bred with an Orpington cock, 448 from four Leghorns injected with Orpington testicles and 240 from two control Leghorns, 308 eggs from two Orpington hens bred with a Leghorn cock, 591 from five Orpingtons injected with Leghorn testicles and 428 from two control Orpingtons, finally 218 eggs from eight F_1 hens *ex* ♀ Orpington \times ♂ Leghorn and 857 from ten F_1 hens *ex* ♀ Leghorn \times ♂ Orpington.

DESCRIPTION OF THE OBSERVATIONS.

The account of the present observations is based on the curves in Figs. 1 and 2, representing the general averages of colour intensity of eggs from all hens of each material. The results obtained in this way should be compared by the reader with the individual averages for separate hens, plotted in Tables I and II. From these it will become evident that the general results cannot be reduced or cancelled by certain fluctuations of the individual averages. The first average intensities of colour are computed for eggs laid by the hens of separate material from April 1st till May 12th, 1923, i.e. till the day on which testicles from a cock of a different breed were injected into fowls of one series of experiments. The following average data refer to the period from May 12th till July 1st, 1923, the remaining being always calculated for full two month periods. In my materials *xenia* would consist in the darkening of the white eggs from Leghorns kept with an Orpington cock

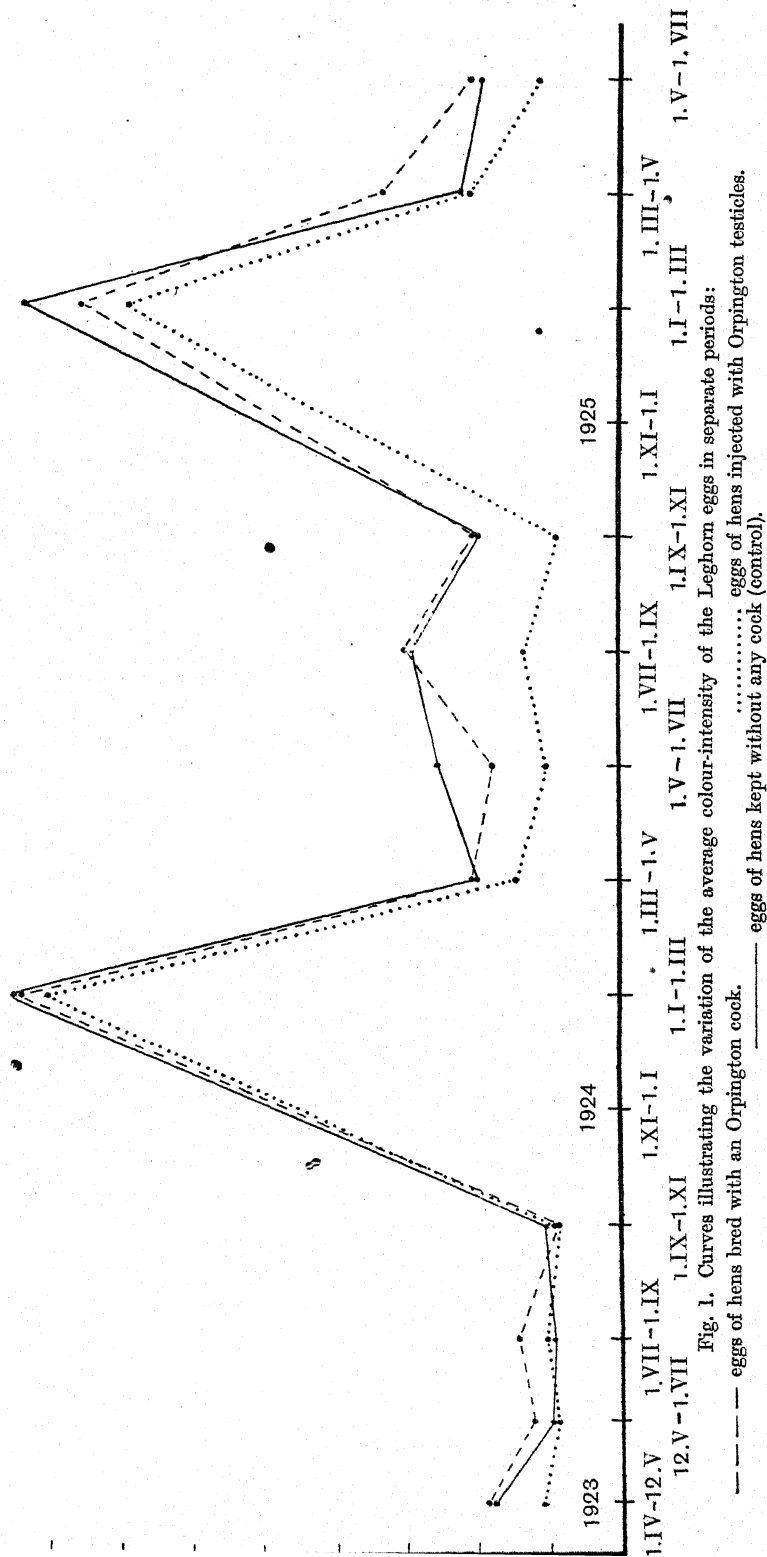
and in the lightening of the brown eggs from Orpingtons mated with a Leghorn male.

A. Observations on Leghorns.

From the curves in Fig. 1 it is evident that in Leghorn fowls permanently bred with an Orpington cock the variations of egg-colour, throughout the period of the observations (cf. Fig. 1, interrupted line) in general exactly correspond not only with those found in the egg-colour of similar hens injected with testicles of a different breed (dotted line), but also to those of the control specimens kept without any cock at all (continuous line). In all three cases the average colour intensities decreased from spring to autumn 1923 and (after a certain interval in the laying period) underwent a very remarkable increase in winter, i.e. from January 1st till March 1st, 1924. Shortly after this increase the colour intensity underwent a marked decrease in all three groups simultaneously, i.e. from March 1st till May 1st, 1924. After this period no large changes in the egg-colour were noticed until the winter of 1924-25. The increase of colour intensity during the winter and the decrease during the spring of 1925 were again very distinct in all three groups.

Certain small inevitable differences in the course of these tint variations between the three groups of Leghorn fowls do not offer evidence of any regularity in the different groups, whether experimental or control. The average colour intensity was, it is true, lower in the control eggs than in the experimental material during the period between July 1st and September 1st, 1923; but from September 1st till November 1st, 1923, and during the periods from January 1st till March 1st and from May 1st till July 1st, 1924, as well as from January 1st till March 1st, 1925, the eggs from control hens averaged a rather darker tint. In the remaining periods the colour of the control eggs was intermediate.

On surveying the seriations of the egg-colour intensity I remarked that the darkest egg, denoted by the 13th, i.e. the highest degree of the scale, was laid during the period between January 1st and March 1st, 1924, by one of the fowls which had been injected the preceding year with Orpington testicles. In the material of Leghorn fowls mated with an Orpington cock as well as in that of the control there was not one instance of such extreme winter darkening of egg-colour. This might, of course, suggest the supposition that we have to do here with the phenomenon of xenia. But, apart from this exceptionally dark egg, the same injected fowls contained two eggs of the first and second degree of



the set of colour, i.e. four eggs which were on the contrary lighter than any egg of both the remaining materials during the mentioned period. Moreover an analogous extreme colour deviation was observed in the control material and it amounted here to the eleventh degree of the scale, being therefore only two degrees lower than the darkest egg of the injected hens. (In the winter period of 1925 the darkest egg was, just the reverse, found in the control material.) This proves that no special character of xenia ought to be attributed to the exceptionally dark coloured egg of the injected hens, notwithstanding its most extreme

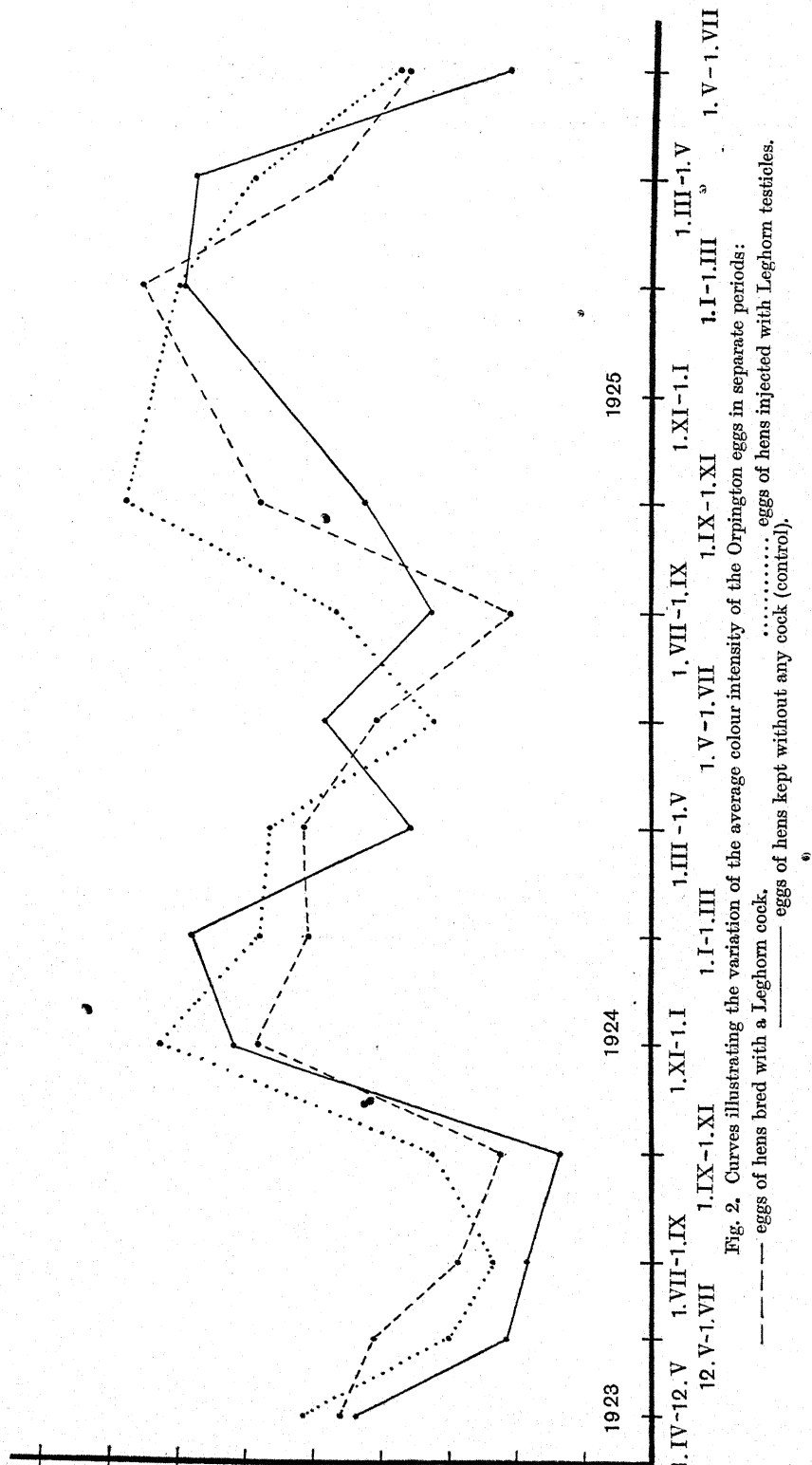
TABLE I.

Individual averages of colour intensity of the Leghorn eggs calculated for each hen separately, in degrees on the used scale.

Period	Hens bred with an Orpington cock. Number referring to each hen					Hens injected with Orpington testicles. Number referring to each hen				Hens kept with- out any cock (control). No. referring to each hen	
	44	45	46	47	48	31	32	33	41	42	43
1. iv. -12. v. 1923	1.67	1.75	2.20	2.00	2.01	1.78	1.57	1.39	1.50	1.94	1.71
12. v. - 1. vii.	1.27	1.58	2.00	1.75	1.83	1.56	1.61	1.29	1.38	1.92	1.18
1. vii. - 1. ix.	2.00	1.85	1.65	1.31	1.86	1.57	1.35	1.64	1.56	1.38	1.50
1. ix. - 1. xi.	1.56	1.25	1.67	1.17	1.40	1.38	1.42	1.62	1.45	1.57	1.50
1. xi. - 1. i. 1924	—	—	—	—	—	—	—	—	—	—	—
1. i. - 1. iii.	4.33	—	6.50	—	—	4.50	—	8.25	3.00	5.57	4.80
1. iii. - 1. v.	2.58	1.92	1.69	2.63	2.00	1.73	2.00	1.00	3.00	2.29	1.94
1. v. - 1. vii.	2.13	2.27	1.50	1.00	1.75	1.27	1.72	—	1.43	2.28	2.36
1. vii. - 1. ix.	3.33	2.36	2.08	—	3.17	1.40	2.00	—	1.60	2.30	2.69
1. ix. - 1. xi.	1.50	1.50	3.83	1.00	1.50	1.00	1.75	—	—	2.00	2.00
1. xi. - 1. i. 1925	—	—	—	—	—	—	—	—	—	—	—
1. i. - 1. iii.	5.40	5.50	5.25	3.20	4.25	5.00	4.75	—	3.60	5.60	4.75
1. iii. - 1. v.	2.20	2.91	2.77	2.25	2.80	1.33	2.00	—	2.60	2.67	1.80
1. v. - 1. vii.	2.50	2.44	1.80	1.67	1.50	1.50	1.33	—	2.00	2.00	2.00

colour darkening¹. Stress must be laid on the fact that during the period from January 1st till March 1st, 1924, the average colour intensity of the eggs from injected hens was just the smallest, since it only amounted here to the fifth degree, being in Leghorn fowls bred with an Orpington cock 5.20, and in the control hens 5.25. On reviewing the individual averages (Table I) we see, it is true, that the highest winter intensity of

¹ As to the especially dark eggs of Leghorns it must be remarked that their deep tint was caused by the fact that they were laid after an interval of from two to three months. All my Leghorns (which have been imported from England) did not lay eggs at all during long periods in winter. On the other hand, the eggs of Leghorns, although being chalk white, as a rule, show from time to time a certain yellowish nuance (cf. table of colour in Benjamin's paper (1)). I believe the pigment became accumulated in such quantities during the long period when the hens laid no eggs that it caused the especially dark pigmentation of the eggs which were first laid after the long winter interval.



egg-colour (8.25) appeared in a hen with injected Orpington testicles (hen No. 33), but at the same time, from January 1st to March 1st, 1924, another hen of the same experimental material laid, on the contrary, the lightest eggs (hen No. 41 with an average egg-colour intensity = 3.00).

From the above it results that no such regular changes of egg-colour appeared in any of the experimental material, as might be attributed to the phenomenon of xenia.

B. Observations on Orpingtons.

Analogous observations were made on the Orpington eggs, viz. in all the three groups the egg-colour becomes lighter during the period between April 1st till November 1st, 1923, whereas in winter from November 1st, 1923, till January 1st, 1924, a considerable darkening was ascertained in hens mated with a Leghorn sire (cf. Fig. 2, interrupted line), as well as in hens injected with Leghorn testicles (dotted line) and in the control birds (continuous line). In the succeeding period of 1924 the pigmentation became gradually lighter (irrespective of certain fluctuations), whereas during the next winter, 1924-5, it became darker again. Here also the course of all these changes does not indicate the appearance of xenia, i.e. of an especial decrease of colour intensity in the experimental eggs, notwithstanding certain differences between the separate groups. And, although the tint of the control eggs was the darkest from January 1st to March 1st, from May

TABLE II.

Individual averages of colour intensity of the Orpington eggs calculated for each hen separately, in degrees on the used scale.

Period	Hens bred with a Leghorn cock. Number referring to each hen		Hens injected with Leghorn testicles. Number referring to each hen					Hens kept without any cock (control). Number referring to each hen	
	16	18	1	6	14	15	20	3	17
1. iv. - 12. v. 1923	10.58	11.09	10.94	11.30	11.50	11.20	10.67	10.73	10.67
12. v. - 1. vii.	10.75	10.45	9.59	10.56	9.46	10.71	9.67	9.63	9.65
1. vii. - 1. ix.	10.42	9.50	8.76	10.12	9.50	10.73	10.00	9.50	9.32
1. ix. - 1. xi.	11.08	8.53	8.48	12.39	9.62	11.10	9.50	8.67	9.50
1. xi. - 1. i. 1924	—	11.40	13.00	11.88	—	12.73	11.92	11.67	11.50
1. i. - 1. iii.	12.00	10.63	10.00	11.80	11.00	11.83	10.50	12.80	11.29
1. iii. - 1. v.	12.23	9.31	9.88	12.10	10.50	11.65	8.50	10.12	10.40
1. v. - 1. vii.	10.88	9.91	9.07	11.64	9.00	11.50	—	10.83	10.95
1. vii. - 1. ix.	10.25	8.38	9.63	10.00	9.00	11.81	10.00	10.00	10.31
1. ix. - 1. xi.	11.33	11.50	—	12.57	—	12.17	—	12.25	9.94
1. xi. - 1. i. 1925	—	—	—	—	—	—	—	—	—
1. i. - 1. iii.	12.43	12.00	—	12.75	11.00	12.57	—	12.67	11.00
1. iii. - 1. v.	11.47	10.23	—	12.20	9.78	12.57	—	12.60	10.50
1. v. - 1. vii.	10.80	9.75	—	10.33	9.67	11.50	—	11.00	9.50

1st to July 1st, 1924, as well as from March 1st till May 1st, 1925, these eggs became the lightest tinted during other periods (e.g. from March 1st till May 1st, 1924, or from September 1st, 1924, till March 1st, 1925). Cf. here also the individual averages for each of the examined fowls separately, plotted in Table II.

C. Observations on F_1 hens.

The eggs of the F_1 generation of the cross between Leghorns and Orpingtons have a typical intermediate tint between the white and the brown colour of both parental breeds. In Leghorns run with an Orpington cock since 1923 the mode of the curve of variation for egg-colour

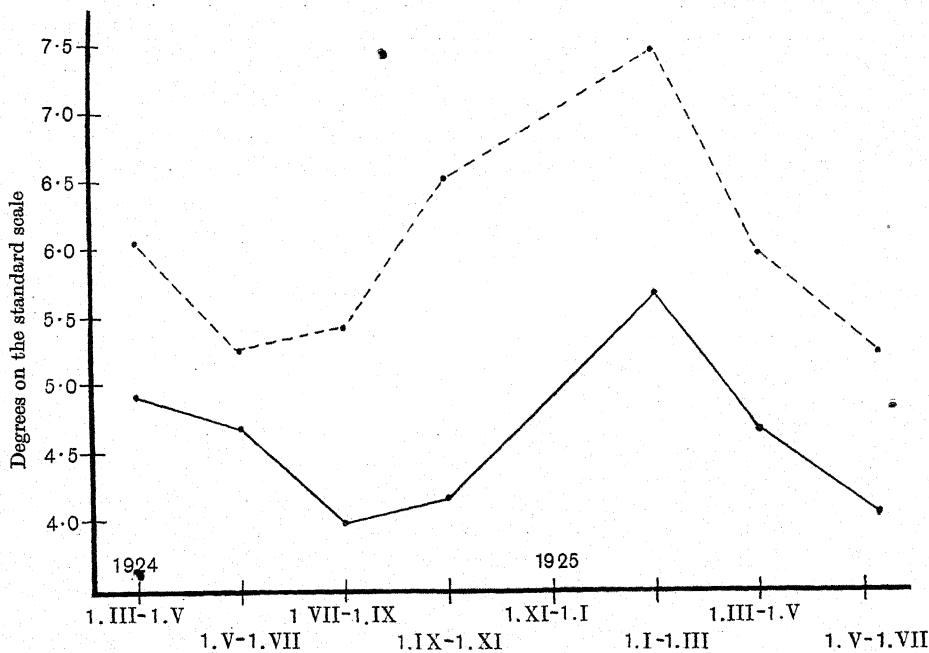


Fig. 3. Curves illustrating the variation of the average colour intensity of the F_1 hens in separate periods:

— eggs of hens from ♀ Orpington × ♂ Leghorn.
 - - - eggs of hens from ♀ Leghorn × ♂ Orpington.

falls on the first or second degree of our standard scale, according to the period of the year in which the observations were made. Similarly in Orpingtons run with a Leghorn cock the position of the mode varies between the 10th and 13th degrees, according to the season. In F_1 birds *ex* ♀ Orpington × ♂ Leghorn the mode varies between third and sixth

degrees and in F_1 birds *ex* ♀ Leghorn \times ♂ Orpington between the fourth and seventh degrees. And while the general average colour intensity ranged in Leghorns between the 1.43 and the 5.20 degree on our scale, in Orpingtons between 9.63 and 12.33, the intensity in the first cross ranged from 3.97 to 5.69, in the second from 5.25 to 7.48, being therefore in both cases more or less intermediate. The individual fluctuations of the average tints of F_1 eggs from separate F_1 fowls were not large enough to cancel the intermediate character of the colour of eggs of this generation. The F_1 hens were one year old at the beginning of the observations, while those of both *P* breeds were already two years. As, according to Benjamin⁽¹⁾, the tint of eggs in fowls undergoes certain changes with age of the birds, I want to remark that in my material these changes are not distinct enough to have any influence on our general conclusion as to the average tint of eggs from hybrids. More detailed data are given in another paper on the inheritance of egg-colour in these crosses (Kopeć^(sa)).

Stress must be laid on the fact that during the winter period (from September 1st, 1924, till March 1st, 1925) a considerable increase of average colour intensity appeared also in the eggs from F_1 hens of both crosses, which was succeeded by a marked decrease in spring (cf. Fig. 3).

GENERAL DISCUSSION AND SUMMARY.

From the above it follows that the changes of egg-colour observed among Leghorn fowls kept with an Orpington cock, or among Leghorns injected with Orpington testicles, are definitely parallel to those which take place in eggs from control hens, to which foreign sperm had not been introduced either by copulation or by injections. The same is true of the egg-colour variability in the experimental and in the control material of Orpingtons. In both breeds it is quite evident that a decrease of egg-tint occurs in all hens without exception from spring till autumn, and a succeeding increase during winter. We have seen that analogous changes are observable also among the eggs of the F_1 generation of the two crosses between Leghorns and Orpingtons. Such behaviour of the eggs from hybrids confirms the opinion that the changes noticed in the experimental material are always brought about by natural, physiological periodical variation. This result is in agreement with the extensive investigations of Benjamin⁽¹⁾, who ascertained on numerous White Leghorns that in these fowls there is yearly "a tendency for the eggs produced to gradually become whiter during the first five or six months of production, and then to become more tinted again toward the end of the production season." Indeed,

my curves on the whole conform with those of Benjamin's White Leghorns (Benjamin⁽¹⁾, Figs. from 36 to 39). Punnett and Bailey⁽¹⁴⁾ also noticed that eggs laid in March are sometimes several degrees darker in colour than those laid in July.

If we consider that no other or more distinct changes, than those characterising normal variability, appeared in hens mated with a cock of a different breed, nor in those injected with testicles from a foreign race, we must deny any possible influence to xenia. It could, of course, be supposed that the poultry material chosen for my research was not adapted to induce xenia. It was not *a priori* excluded that the brown egg-colour of my Orpingtons, as well as the specific white pigment of eggs from Leghorns, which according to Wickmann's⁽²¹⁾ research is characteristic of white eggs; could, owing to purely chemical causes, be unable to undergo such mutual changes. The typically intermediate egg-tint in hybrids proves, however, that the colours of these eggs may exert an influence on each other by means of genetical mingling.

Consequently I am inclined to look upon the previously described xenia in fowls as based upon a misunderstanding due to insufficient consideration of natural variability of egg-colour intensity. I believe that v. Tschermak's⁽¹⁸⁾ well-known work may also be explained by this variation. It may be objected by the adherents of xenia that, while in my observations on natural variability the brown as well as the white egg-colour undergo the same changes (i.e. simultaneous increase or simultaneous decrease of intensity) during the same seasons, the contrary is true of the experiments of v. Tschermak: during one and the same period of observations white eggs from certain breeds become darker and the brown eggs from other breeds lighter. I would remark, however, that v. Tschermak's results are unfortunately recorded in too general a manner to allow of an exact analysis. It must be emphasised that such results as those obtained by v. Tschermak may have been fortuitous. First of all it is remarked by this author that in two of the six cases he describes a change was hardly visible, i.e. distinct changes took place only in four hens belonging to various breeds. It is evident from my Tables I and II that the individual fluctuations of egg-colour intensity are often very distinct, and that in one and the same groups of fowls the increase of the colour intensity during autumn and winter and the decrease during spring and summer begin earlier in one specimen and later in another, lasting differently in separate fowls. It is also possible that in v. Tschermak's case the opposite changes of egg-tint during the long period of hybridisation, lasting nearly a year, were

influenced not only by the ages of the individual hens, in respect to which no data are given, but also by the very different frequency of egg laying in different birds. This is the more probable because, as my own inquiries show, egg-colour intensity seems in some degree to depend on the more or less frequent function of the sexual tracts, i.e. to their greater or smaller widening during separate laying periods (Kopeć(8)). It is probable that a division of v. Tschermak's series of observations into shorter periods would to a considerable degree efface the opposite changes he describes. v. Tschermak emphasises moreover a distinct increase of colour variability, observed as a rule during bastardisation, which, according to his view, is very characteristic of the period of xenia. From my observations on natural, periodical egg-colour changes in fowls the increased variability during hybridisation periods, lasting almost a whole year, as compared with the much shorter periods of pure culture in the experiments of v. Tschermak, is quite clear, and there is no need to attribute it to crosses with a foreign cock.

Nor do I now consider that importance can be attached to my earlier observations on Polish Greenlegs (Kopeć(6)). In these experiments Greenleg hens mated in 1919 with a cock of the same breed laid cream eggs of an average colour intensity 7.1 during April and May, whereas mated the next year with a Leghorn they produced during the same months much lighter eggs, i.e. of an average colour intensity 5.4 degrees on our scale. Considering the normal periodical variation of egg-colour in fowls, this difference might however have been exclusively fortuitous: owing to certain differences of egg-colour in the same months during separate years, the natural spring decrease of colour may have appeared earlier in 1920 than in the preceding year. This happened indeed during my present investigations: it may be observed in Figs. 1 and 2 that the average changes of egg-colour in separate months of the year 1924 were somewhat different than during the similar periods of the years 1923 or 1925.

In conclusion I want to lay stress on the fact that the phenomenon of xenia could not *a priori* be considered merely as a prejudice of numerous breeders but deserved careful examination. Little is yet known as to the nature of the pigments of the egg-shell, and students of genetics cannot therefore summarily reject the supposition that the resorbed sperm of a foreign breed may introduce into the female organism certain special substances. It was conceivable that the sexual duct might be "impregnated" by these substances, so that the eggs laid by the hens under examination would exhibit changes characteristic of xenia (cf. analogous considerations on telegony, Kopeć(7) and Löhner(10a)).

From the above inquiry we may draw the following conclusions:

1. The "white" eggs of the Leghorns, as well as the brown Orpington eggs, exhibit a distinct periodical variation of colour intensity. The intensity of pigmentation decreases from spring till autumn and increases during winter.
2. No other changes of egg-tint have been observed in the hens of both the breeds examined on interchanging the cocks.
3. Even repeated injections of Orpington testicles into Leghorn hens or Leghorn testicles into Orpington fowls did not cause any changes of egg-shell tint in the direction of the egg-colour of the breed of the cock used for the injection.
4. The egg-shell colour of the F_1 fowls of the cross ♀ Leghorn \times ♂ Orpington or *vice versa* is intermediate between the parental breeds.
5. The earlier observations on xenia in birds cannot be considered as decisive on account of various methodical inaccuracies. The appearance of xenia remarked by various authors may be fully explained by the misunderstanding caused by insufficient knowledge of the normal periodical variation in egg-colour.

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THE COAT OF THE MOUSE (*MUS MUSCULUS*).

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(With Four Plates, Ninety-one Text-figures and Twelve Charts.)

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INTRODUCTION.

THE study of mammalian hair was taken up on the suggestion of Professor W. Garstang "as a basis for breeding and other experiments." A number of investigators have already done work on the coats of various mammals in the hope that their results might prove useful to Genetics. Many contributions of this kind have had reference to the fibres of the large domestic animals, especially sheep. Nathusius and McMurtrie were working with this aim some time before the close of the last century, while more recently there have been the researches of Ewart and Crew with his associates in Britain, and Kronacher and others on the Continent. During the last few years a good deal of attention has been paid to the details of structure and pigmentation of the hairs of the laboratory rodents. Werneke and others have examined the pigment of different colour types of the Mouse (*Mus musculus*). On *Peromyscus* there is the work of Sumner, Collins, and Heustis. Salaman and Wucherer have reported on the inheritance of structural

characters in the Rabbit, both being largely occupied, in different ways, with the Angora coat. Bodmer-Giger's comparison of *Lepus europaeus* and *Lepus varronis*, which includes a statistical examination of the fur, has bearing on a number of matters to be dealt with in this paper. Explanations of the way in which colour factors produce their effects in the coats of rodents have been put forward by Onslow and Wright.

In this account only brief references are made to a number of books and papers on hair development and structure that have proved very useful, and much important work is not mentioned. Full bibliographies will be found in several of the papers that are cited. In studying development the most help has been received in this particular investigation from the work of Oyama on the albino Mouse and of Segall on the Guinea-pig, and in working on cuticular scales from Hofer and Hausman. De Meijere is, of course, the pioneer on hair arrangement, and in the recognition of hair types all who have followed him are indebted to Toldt.

Much of my work was done before I became conversant with some of the researches on the coats of rodents that have been mentioned, but there has been little overlapping. Wild mice being difficult to breed in confinement, mutant types have mostly been used, especially chocolate animals having one parent chocolate and the other albino. The main series of skins consisted of about seventy chocolates carrying albinism (Cc) of known ages up to four months. The first part of this paper is concerned with the hair types, especially those of the first pelage or "hair generation," of the main area of the body. The hairs of this pelage usually complete their growth at about three weeks, each standing alone in its own follicle. The second part deals with hair development and succession and related matters. New terms have mostly been suggested by Professor Garstang. Methods are described in an appendix.

I. THE HAIR TYPES.

1. *General Description.*

Most attention has been paid to hairs from the "mid-dorsum," that is, from the middle line half-way between the ears and the root of the tail, and the detailed studies have been made on hairs of the first pelage. Apart from some descriptions of cuticular scales nothing will be said in this part of the paper about hairs from outside the main area from small areas to be termed anomalous areoles. These are the circum-oral and sub-caudal areoles and those of the ears, the margin of the eyes, the tail, and the feet. Vibrissae and hairlets (p. 317) as well as a few special

hairs round the mammae are also not dealt with until Part II. The hairs of *Peromyscus* and *Mus musculus* are very much alike so that only a few general features have to be considered as a preliminary to the description of the several hair types.

The whole of a hair lies in one plane apart from a small number of hairs of the underfur possibly distorted by accident. Throughout nearly all the main area the hairs project from the skin at a rather small angle, the hairs sloping directly or more or less caudad. Viewed at right angles to the plane in which it lies a hair shows a characteristic curvature. These curves may be observed in hairs free from pressure either dry or immersed in liquid. Examples are shown in camera lucida tracings (Figs. 43 to 52) and in some of the low power photographs of entire hairs. In hairs on the mid-dorsum the convex side of the long bend is ectad and cephalad, and that surface will be called ectal, the opposite surface ental. In those overhairs that have a constriction the bend is specially pronounced. Another much slighter bend can often be observed in hairs of all types near the extreme apical end, the concave side of this little curve being ectad. The tips of hairs are very slender but this bend has been noticed very frequently.

The cross-section of any hair is more or less circular near the two extremities, but the broad parts of most overhairs are far from cylindrical. The long axis is then at right angles to the plane of the hair, an overhair of the mid-dorsum presenting its greatest breadth when viewed from the head or tail. From the middle of the flank one would view what may be called the side of the hair. In many overhairs the ectal surface is concave in transverse sections, the ental convex (see Bodmer-Giger, pages 57 and 58, and the figures on pages 62 and 65), and there are then characteristic differences between the two surfaces in the shape of the cuticular scales. In the hairs of the underfur, which have a zigzag form, the convex surface of the distal bend faces towards the head, and consequently that surface, which does of course become concave at the next bend, and so on, is called ectal.

The units of the axial region, which in coloured hairs contain masses of pigment, are called septa when complete, septules when incomplete, the adjectives septate and septulate being used. In III- or IV-septulate hairs the units attached to the sides of the hair are called outside, those between them, inside septules.

The term tip is applied to the solid apical region, but within the basal end of the tip are sometimes included a few "apical vacuoles" separated a little from the uninterrupted series of vacuoles of the shaft (Fig. 79).

On the mid-dorsum the solid base of the hair that ends in a little club is always short.

A lock-out has always been kept for abnormalities of hair structure, but they are rare, and it will scarcely be necessary to say anything about them in the present paper.

The skins were each given a number and a letter, as 25 *a* or 54 *c*, the number signifying the age of the animal in days, the letter distinguishing between any mice of the same age.

2. *Major Features.*

The following key serves to distinguish the hair types of the first pelage from the mid-dorsum. With little alteration it could be used for hairs from other parts of the main area or for those of later hair generations.

- | | |
|--|---------------------|
| 1. Shaft without a constriction (Types A, A-B, B) | See 2 |
| Shaft with one constriction (Types B-C, C) | See 4 |
| Shaft with more than one constriction (Types C-D, D) | See 5 |
| 2. Tip at least .060 cm. in length (Types A, A-B) | See 3 |
| Tip shorter than .060 cm. | Awl or Type B |
| 3. Shaft nowhere III-septulate | Monotrich or Type A |
| Shaft III-septulate at some point | Type A-B |
| 4. Constriction II-septulate throughout | Type B-C |
| Constriction containing at least one complete septum | Auchene or Type C |
| 5. Shaft II-septulate at some point | Type C-D |
| Shaft septate throughout | Zigzag or Type D |

The overhairs include Types A to C, the typical underfur consists of Type D, the scarce Type C-D being transitional. The proportions in which the first pelage types occur on the mid-dorsum are roughly: A and A-B 2 per cent., B and C 14 per cent., D 83 per cent., B-C and C-D together less than 1 per cent. A is found in somewhat larger numbers than A-B. B may be more plentiful than C or it may be scarcer. In the description which now follows it does not seem necessary to attempt to mention all the characteristics of hairs or parts of hairs which may be observed in the photographs and diagrams. Nor does it seem useful for the present purpose to submit statistical tables, but a table of measurements is given for the representative hairs from which drawings of the cuticular scales are reproduced.

Monotrichs or Type A. The name has been chosen because hairs of this type belonging to hair generations later than the first always stand alone in the follicle when fully grown (p. 307), whereas a club-hair of any other type may stand in the same follicle as another full-grown hair. These hairs are evenly distributed in the skin and their tips project

above the rest of the coat. The length is of the order 0.9 to 1.0 cm. The tip may be more than a quarter of a centimetre long; apical vacuoles are often present. There is more variation in the length of the tip than in that of the rest of the hair. The greatest breadth of the shaft is less than that of the broadest hairs of Types A-B and B. The cross-section of the broad parts is approximately circular in the longer hairs, or somewhat oval. Often the longer hairs have a thicker cortex combined with a smaller maximum breadth than the shorter ones, so that the medulla tends to be narrow in the long hairs, and then the numbers of septules in the axial units are fewer. It seems likely that monotrichs are tactile in function. They belong to the Leithaare of Toldt's classification, as distinct from the Grannenhaare or smaller overhairs.

Awls or Type B. These hairs measure from about 0.6 to 0.7 cm. in total length, the tip usually from 0.03 to 0.04 cm. Apical vacuoles occur only occasionally. The cross-section in the broad parts is bean-shaped, or concavo-convex. The cortex is thin. Septules are numerous, many hairs being IV-septulate at the broadest part. In awls from the mid-dorsum of the same animal the range of variation in a character is comparatively small. Well-marked differences have been found between the assemblage of hairs of this type from the mid-dorsum of two animals of the same sex.

Auchenes or Type C. This type differs from the last in having a septate constriction of oval cross-section at about two-fifths of the length of the hair from the apical end. This lies between the distal "blade" and the proximal "stalk." Auchenes are on the whole a little smaller than awls. Apical vacuoles are very rare.

Zigzags or Type D. On the mid-dorsum these hairs have so far been found to have either three or four distinct constrictions, while just above the base is the "basal curl" suggestive of a tendency to form either one or two additional constrictions. Viewed from the side the outline of the hair is a damped harmonic curve. Zigzags from the mid-dorsum vary in length considerably. The longest are of similar length to average awls or auchenes, the shortest about three-fifths as long. The proportionate numbers of zigzags of different lengths, and the relative length of the several segments promise to be worth detailed study. For the form of the various parts reference may be made to the cuticular scale diagrams. The cross-section is more or less circular save at the constrictions where it is flattened in different degrees. The tip is always short, from little more than 0.02 cm. to about 0.035 cm. Apical vacuoles occur very occasionally.

On parts of the body where the hairs are shorter, zigzags are found

with fewer bends, that is, with two constrictions or one, together with the basal curl. Shorter hairs differ from longer ones in two ways, in the smaller number of the segments, and in the shorter length of the individual segments. Where the hairs are very short, as just behind the mouth, the single constriction and the basal curl may approximate closely, while in some only the curl, and this sometimes faint, serves as a mark of Type D. Finally, still shorter hairs, in which one fails to make out the curl, may be regarded as the lowest common denominator between underfur and overhair.

Intermediate types, A-B, B-C, and C-D. Type A-B has been separated from Type A by the presence of septules, and from Type B by an arbitrary length of tip, because these distinctions have proved convenient ones on the mid-dorsum. As the figures will indicate, there are many features in which the transition from monotrich to awl can be traced. In later hair generations it is difficult to draw a line between Type A and Type A-B. In parts of the coat where the hairs are shorter and less broad the differences between the overhair types become less, and Types A and B are the first to merge into one another.

Type B-C is defined on the mid-dorsum as having a perceptible narrowing in the shaft in the region corresponding to the constriction of an auchene, but with that part of the shaft septulate throughout. As a hair with a single complete septum in that region is classed as Type C the rarity of this intermediate type is not surprising.

Although Type C and Type D differ widely in that the former is septulate and has only one constriction, the latter entirely septate with several constrictions, transitional forms in all hair generations examined are strikingly scarce. On the mid-dorsum the majority of these intermediate forms have three or four constrictions and the basal curl, and have a small number of septules in the first or distal segment. Hairs with two constrictions and no basal curl are especially rare. When the distal segment is broad, resembling the blade of an auchene, it has a concavo-convex cross-section. A few hairs with one constriction have been found in the first pelage on the dorsum of a wild mouse, having the distal region like the apical segment of a zigzag, and the long proximal region like the stalk of an auchene. Transition forms between overhair and underfur have already been described from close to the mouth; other forms have been found near the ear and tail.

It has thus been shown that a complete transition in hair form exists between monotrich and zigzag, and that connecting links join up all the ordinary hairs of the main area. It may further be stated that from what

has been learnt of the special hairs that are to be dealt with later, it is believed that with all the diversity in the coat of the Mouse, every hair on the entire animal would find a place in a system showing no sharp breaks.

The work on the major features of the hair types that has now been described was intended to afford a basis for comparisons between the hairs of different individuals. For this purpose the awl is likely to be the most convenient type. It is present in fair numbers; being without a constriction it is of simpler form than the auchene; the variation within the type is not very wide, and for the features that lend themselves to measurements and counts it is easy to ascertain the most usual numerical index; in consequence of their flattened shape the hairs are easy to orientate on a broad face; and the medulla shows clearly through the thin cortex. For some purposes the zigzag might have advantages; it is septate throughout, can be placed on its side instantly, and is very numerous. For any hair type the characteristics most likely to be of value can be chosen. A numerical index for each of a small number of features is sufficient to give one a very fair picture of any hair. This is given in the awl by the total length, tip length, maximum breadth, and number of inside septules. It may be added that preliminary studies have shown that the hair types occur in different proportions on the same part of the body in the first pelage of different individuals.

Some examination has been made of hair arrangement on the mid-dorsum. It will suffice to refer to Figs. 10, 11, 12, and 22.

3. *Cuticular Scales.*

This section deals with the shape of the apical edges of the cuticular scales examined in hairs not subjected to any disintegrating process. The hairs of the main area from which drawings were made all belonged to the first pelage, but the scales of the same hair type are essentially the same in each of the first three hair generations.

The region of the shaft shown in a drawing is indicated in a simple way by the use of a fraction. This—say $3/10$ or $19/20$ —shows the approximate part of the total length of the shaft, exclusive of the base, which lies distal to the position in question, and it is convenient to speak of Position $3/10$ and so on. The scale margins are drawn in a heavier line than the outlines of the septa and septules, which, being objects of appreciable thickness, are represented somewhat diagrammatically. Pigment is generally entirely omitted from the diagrams; something of its distribution in the medulla and cortex may be seen in some of the

photographs. The scale edges are drawn, as Hausman puts it, "on the single plane of the paper, as though the hair had, by some means, been crushed out flat without distorting its structure." Where figures are given for both the ectal and ental surfaces of the same region it is generally possible to make out how each scale edge which runs to the margin of one figure is continued on the other. This is most easy where adjacent scale edges are far apart and the cross-section is not circular. The flatter the cross-section the more completely can the scale margins be represented in the pair of figures. Table I gives measurements and other particulars for hairs from which figures of scale margins are taken.

TABLE I.

Data for hairs from which drawings of cuticular scales are given.

Hair No.	Kind of hair	Mouse No.	Part of body	Length of tip cm.	Length of shaft* cm.	Length of entire hair cm.	Max. breadth μ	Medulla†		
								I	II	Inside septules
15	Type A	25 a	Mid-dorsum	.19	.73	.94	26	882	12	None
191	Type A-B	25 a	Mid-dorsum	.14	.75	.90	30	852	263	1
195	Type A-B	25 a	Mid-dorsum	.13	.67	.82	33	756	—	27
57	Type A-B	25 a	Mid-dorsum	.067	.63	.72	42	694	—	156
54	Type B	25 a	Mid-dorsum	.036	.61	.67	38	627	—	113
194	Type B	25 a	Dorsum (post. part)	.028	.64	.68	30	—	—	23
10	Type B	20 a	Mid-dorsum	.033	.60	.65	30	—	—	55
46	Type B	25 b	Mid-dorsum	.042	.65	.71	36	—	—	169
5	Type B	25 b	Mid-dorsum	.034	.57	.62	30	—	—	30
30	Type B	25 b	Mid-dorsum	.037	.58	.64	29	—	—	4
001	Special hair	25 b	Lower lip	—	—	.045	14	No medulla		
02	Special hair	20 a	Ear	.028	.046	.08	25	47	12	None
197	Special hair	25 a	Tail	.063	.066	.15	35	69	9	None
W 5	Vibrissa	25 c	Lip	.71	.66	1.39	65	Some II present		
M 1	Hairlet	20 a	Mid-dorsum	—	—	.035	6	No medulla		

* Base excluded.

† The particulars are not equally complete for every hair. I=units attached to one side. II=units attached to one side that are opposite to similar units attached to the other side.

Drawings of the scale pattern are also given from the following hairs from the mid-dorsum of No. 25 a:

No. 106, Type C. Tip length .032 cm. Blade, length .24 cm., maximum breadth 30 μ , 79 inside septules. Constriction composed of 41 sepa, length .028 cm., minimum breadth 15 μ . Stalk, length .33 cm., maximum breadth 24 μ . Total length including base .65 cm.; 673 I in entire hair.

No. 46, Type C. Tip length .031 cm. Blade, length .26 cm., maximum breadth 34 μ , 80 inside septules. The constriction is taken to be the narrowed region bounded by the most distal septum and the most proximal septum, this part being .019 cm. long and containing 23 I and 12 II, with minimum breadth 20 μ . Stalk, length .033 cm., maximum breadth 27 μ . Total length including base .66 cm.; 637 I in entire hair.

No. D 1, Type D. Length of parts, Tip .028 cm., Segment I .094 cm., Segment II .140 cm., Segment III .136 cm., Segment IV .116 cm., Base .024 cm. Maximum breadth, measured from the side, 15 μ , this measurement being found in Segments II, III and IV. Total length .54 cm.; 499 I in entire hair.

The other Type D hairs chosen were very similar to No. D 1.

The simplest kind of scale-margin pattern is annulate, consisting of a series of simple rings each encircling the hair. This is characteristic of all parts of very small hairs that are circular in cross-section and have no medulla. This pattern is also found in its simplest form at the apex of hairs of every kind. On the base it is generally slightly modified. Both these parts are slim, free from medulla, and circular in cross-section. All other patterns can be regarded as modifications of this very simple one. A gradual transition can be traced from the annulate arrangement on the tip to the region of any hair where the pattern is the most complicated. On some large hairs the pattern nowhere departs much from the simplest kind, and for any corresponding region a series of hairs may be selected which show a transition from the most simple pattern to the most complex. The pattern is often not the same on the ectal and ental surfaces.

Overhairs. The scales of different regions of the same surface of an overhair differ in various ways, notably in the average distance between two adjacent transverse margins and in the degree to which they make the shaft serrate. Between the ectal and ental surfaces of all but some of the largest hairs there is a constant and striking difference in that the grooved region of the ectal face shows a U-pattern which persists without a break for a long distance. On the ental surface the transverse scale margins run more straight across the shaft and at the most a suggestion of a U-pattern is found here and there. Transitions between the simple pattern of monotrichs and the more complicated one of the small overhairs are found on Type A-B hairs. From one of these hairs of intermediate type a very slight U-pattern is figured. Where the ectal furrow is long the U-pattern extends far along the hair; where the furrow is deep the individual U-shaped margins tend to be long. In this connection see especially Figs. 92 to 96. In auchenes the furrow is apt to extend further both distally, on the blade, and proximally, on the stalk, than on the shaft of awls. On narrow constrictions in auchenes there is a U-pattern on the ental as well as on the ectal surface, but away from the constriction the U's soon give place to the ordinary pattern of that part of the ental surface of a small overhair.

Underfur. In the zigzags the pattern throughout is easily derivable from the annulate one. Shallow U's are characteristic of the ectal surface of the first segment. At the constrictions there is a distinct tendency towards a U-pattern on that surface of the hair which is on the concave side of the particular bend, while on the other surface any appearance of U's is absent or more slight. In the transitional form Type C-D the

scale pattern resembles that of auchenes or zigzags according as the hair approaches in major features the one type or the other.

Extremities of hairs. In the longest overhairs the segments of the annulate tip near the point tend to be shorter than in awls and auchenes. The zigzags sometimes have tips that remain very thin for a greater distance than in any overhair examined, yet others have tips that, near the point, are like one or other of the forms usual for the larger types. Very occasionally a scale margin has been found to take a short spiral course round the tip. This has been observed in a few zigzags and hairlets and in a single auchene. On the bases of hairs of all types the scale pattern, as already stated, is on the annulate plan. Various writers have found that scale margins are not to be detected either on hair-clubs or for a short distance above, and the same is true in the Mouse.

Vibrissae. Amongst a number of special hairs from which drawings are produced that show various patterns on the annulate plan mention may be made of the vibrissae. In these hairs there are regional differences in the distances between adjacent transverse margins and in their waviness. In the shorter vibrissae the margins are neither so crowded nor so wavy as in the longer ones. In the vibrissae specialisation would appear to have taken place in the two directions indicated.

II. DEVELOPMENT AND SUCCESSION OF HAIRS.

1. *Hair Development.*

The development of the hairs has been studied as far as was necessary to afford a basis for the work on the succession of the hairs of the several generations. It will be very apparent that a more detailed knowledge of the histology of some of the processes to be considered is much needed, but this could best be obtained in a separate investigation. Hair development has been described very minutely for other animals by Lwoff, Stöhr, Spencer and Sweet, Hofer, Segall, and others; and in the albino Mouse the histology of development of the first pelage has been investigated by Oyama, who paid particular attention to the very early stages.

In the skin of the Mouse it will be enough to distinguish the corium, the Malpighian layer rich in nuclei, and the corneous layer. The follicles of the hairs of the first pelage are developed, as in other animals, by a downgrowth from the Malpighian layer. German writers distinguish four stages in early development, Haarkeim-, Haarzapfen-, Bulbuszapfen-, and Scheidenhaarstadium, the last continuing until the hair becomes a

club-hair. For the present purpose it is convenient to divide the developmental history into the following three phases:

(1) The Anagen phase, the longest part of the growing period, lasting from the initiation of growth until, in coloured hairs, pigment ceases to pass upwards from the bulb at the base of the hair-root; at this point rapid reduction in the size of the root begins.

(2) The Catagen phase, during which the root is decreasing in size.

(3) The Telogen phase, the hair having become a club-hair and not growing further; the medulla-free base terminates in a little club which continues to be surrounded by the small sheath that is formed at the end of the preceding phase.

Anagen phase. In the earliest stage shown (Fig. 13), from the skin of a new-born Mouse, the follicle of a large overhair is in the third of the four stages recognised by Oyama and others. Later stages are shown in the succeeding figures. In the later hair generations, apart from the fact that the new downgrowth is not formed direct from the Malpighian layer of the skin, the process is the same. The papilla appears very early, and the sebaceous glands are first recognised soon afterwards. To each follicle a pair of glands is at any rate the rule. Oyama finds the arrector muscle to be developed at an early stage from a group of mesenchyme cells. The muscle is attached to the persistent region of the follicle at about the level of the openings of the ducts of the sebaceous glands. The basal portion of the root, the bulb, where the pigment is formed, is of somewhat greater diameter than the rest of the root. The full length of the root is attained something like a week after development has started, and this large size persists into the catagen phase. Frequently there is a curve in the root at the apical end of the bulb. Segregation of the pigment granules into groups begins in the bulb near the apex of the cone of pigment. The great part of the pigment formed becomes enclosed in the cortex or medulla of the hair, but a small portion may never pass into the hair; the fate of this will be described shortly. The inner root sheath by its glassy appearance contrasts sharply with the outer, especially in the apical parts where nuclei are absent or scarce. Two layers, to be regarded as those of Huxley and Henle, can be recognised in the inner sheath. The apex is close to the openings of the gland ducts. In the short distance between this point and the surface of the skin, only a single sheath surrounds the hair, which it does not grip at all tightly. In one longitudinal section (Fig. 19) this region of the follicle is flask-shaped, but this is not always so. As in hairs in general, growth

takes place entirely in the root; some time before the apex of the inner root sheath is passed by a part of the growing hair, that portion has acquired its permanent form.

Between follicles of the different kinds of hairs of the main area in the anagen phase there are differences at a corresponding stage in the size of the whole follicle, in the size of the pigment cone, and in the thickness of the inner root sheath. At three days there is a sharp contrast between the bulb of a large overhair, where the long tip is still in course of formation, and that of a small overhair with medulla already being produced. At birth the skin has been pierced by the tips of a few vibrissae and by those of certain shorter hairs near the mouth that are also very likely tactile in function, but young follicles are found throughout the main area as well as on most of the areoles. On the main area they are most numerous dorsally, and on the whole the furthest developed there. On the dorsal surface development is most advanced on the head. In successive positions down the sides, and on the under surface towards the mid-ventral line, the numbers fall off, and the latest stage of development reached becomes earlier. Development is also advanced on the feet on the part belonging to the main area where the hairs are noticeably stout. In many of the bulbs on the dorsum pigment is easily seen. On the venter only a few have pigment, and then only a trace. This is to be attributed only partly to difference in stage of growth, for the hairs on the dorsum are the more heavily pigmented in their apical parts. Most of the early follicles will produce overhairs. At one day I have found the tips of the large overhairs, and of a few of the smaller ones, to have pierced the skin over a large part of the surface of the body. Broadly speaking, hair development starts at birth. On the dorsum I have first recognised underfur follicles with confidence at two days. My belief is that new overhair follicles are not founded after three days on the dorsum, but that the initiation of underfur follicles continues up to about seven days, and on the venter to about nine days. De Meijere, on examining very young animals or embryos, when already conversant with hair arrangement in older specimens, found in a number of mammals that the larger hairs were being formed earlier than the smaller, or were at a more advanced stage. In the follicles of neighbouring zigzags of the first generation any part of a segment, from the middle of the segment to the narrowest part of a constriction, may be in process of formation at the same time. Nor are the similar parts of a segment of neighbouring second generation zigzags always in course of formation at the same time, although the neighbouring hairs begin their development at the same time. This is

to be attributed to the differences observed in the lengths of the same segment in different hairs.

Catagen phase. As the growth of the medulla-containing shaft is completed all the pigment, apart from a small portion in some follicles, finds its way out of the bulb into the growing hair. The hair-root now quickly decreases in size and in something like two days a rapid transformation in the part of the follicle below the glands leads to the final phase. The inner root sheath disappears completely, its absorption beginning at the base and ending at the apex. The fate of the outer root sheath is not so clear. The hair-club can be seen in process of formation while the root is still quite long and a large part of the inner root sheath still remains. The club-sheath makes its appearance round the base of the club. As the root shortens, tissue is sometimes found at the base of the follicle suggestive of a collapsed tube, like the "Haarstengel" of Segall and other writers (Fig. 21). With them the Haarstengel appears to consist of corium tissue, but in the Mouse I cannot satisfy myself that the hair-root has a definite corium sheath, and believe the structure figured to consist of outer root sheath. Very frequently the base of the shortening root does not tail off in this way but remains round, presumably because the absorption of the outer root sheath or the transformation of its material is proceeding sufficiently fast. As the root shortens, the hair-club and its sheath rise, and ultimately reach the level just below the sebaceous glands, at which they remain. The papilla is also raised, and inside the papilla or closely associated with it, and ultimately enclosed in the small region to be called the basal saccule, there is frequently a little "residual pigment." Between the club-sheath and the basal saccule the hair germ appears, destined to give rise to the companion hair of the next generation. The hair germ is usually formed in the later part of the catagen phase, and generally, when the changes that bring in the telogen phase are completed, is of the order of size relative to the other parts of the follicle shown in the photographs (Figs. 20, 24, and others), but occasionally is smaller. Now and again the residual pigment is in contact with the club-sheath, when sometimes I have found the hair germ wedged between the club-sheath and the saccule, but sometimes have not been able to recognise it. All that remains of the original tissue of the transformed region of the follicle I believe to be simply the outer portion of the basal saccule. In various places in the literature it is suggested that the substance of the disappearing root sheaths passes into the hair-club. This and other matters in the description just given call for closer study.

Telogen phase. As in other mammals, after the completion of the club the hair does not grow. Apart from a few exceptional cases there is an interval in which I fail to detect any change in the follicles of the ordinary hairs of the main area until, after a shorter or longer time, the next anagen phase begins. This applies also to most follicles containing special hairs, but not to those that produce vibrissae.

Development of companion hairs. The development of a companion hair begins in the enlargement of the hair germ. Once started, growth is rapid. Those parts of the follicle which disappeared or underwent change in the catagen phase are formed afresh, and a new hair is produced. Early stages are shown in photographs of some of the special hairs (Figs. 34, 35, and 37). The basal saccule takes no part in this development. In cleared skin from the dorsum showing the very early anagen phase of the second hair generation, the connective tissue underlying the follicles not having been removed, the residual pigment is not in compact masses but in scattered groups below the very young bulbs in which the earliest pigment granules are just appearing, and even below hair germs that are only slightly enlarged. This indicates the rupture of the saccules. A new papilla is formed at the base of the follicle. There has been much discussion in the literature, which is reviewed by Segall, whether or not the same papilla can function in the growth of another hair. Some workers have taken one view, some the other. In the Guinea-pig in defined cases Segall has found both modes of development. In the Mouse, in the follicles which produce vibrissae, I have come to the conclusion that another vibrissa begins to be formed as soon as the growth of its predecessor is completed, and that the original papilla persists. In the clubs of the longer vibrissae there is frequently a little pigment (Fig. 28), and in the follicles containing such hairs it is the rule for some pigment to be found in the root throughout the period covering the completion of the growth of one hair and the initiation of the growth of its successor. In follicles that give rise to other special hairs the fate of the papilla, and the manner of development of a successional hair, are at any rate usually the same as in those of the ordinary hairs of the main area, but whether the papilla ever does persist in the follicles of any other special hairs has not been finally decided.

At the level of the gland ducts the new hair enters the persistent part of the follicle, where it is surrounded by the same sheath as its older companion, so that the two hairs come into contact. The telogen phase is reached by the same series of events as before. The new hair germ and basal saccule lie below the club-sheath of the new hair, and almost in-

variably the basal end of the newer hair-club lies at the lower level (Fig. 12). Very occasionally the level is the same. The development of later companion hairs is a repetition of the same process. The hair germ and basal saccule below the club-sheath show which is the newest hair. The basal end of the club of a third generation hair is very nearly always slightly lower in the follicle than that of either of the older hairs, so that the level of the hair-club in the follicle itself is usually sufficient to show to which generation a hair belongs. After a hair has fallen out I have failed to see anything of its club-sheath. Segall quotes Garcia as saying that in Man there is no sign in a follicle to indicate that one hair has been substituted for another.

On the main area, and observations on the areoles point to the same being true there, I am convinced that when all the hairs within any area in which the conditions are comparable have reached the first telogen phase, all later hairs are grown in the follicles already in existence there. No sign has been found of the formation of fresh follicles direct from the Malpighian layer. In the light of a great deal of dissecting I believe that with the growth of each hair generation on the main area a hair is grown in every follicle. This statement does not of course have reference to the special hairs situated within the main area. I have never found more than one hair growing in a follicle at once on the main area, and the same is true for all follicles examined elsewhere with two exceptions, both on the tail of the same animal, which was more than a year old. In one of these two exceptions a hair with its inner root sheath not quite completely reduced had a half-grown companion. In the other both hairs were about half-grown. In both cases the two hairs had separate root sheaths below the persistent part of the follicle.

The history of the several parts of a typical follicle, some persistent, some transitory, may be briefly reviewed. The sebaceous glands, each with its duct and sheath, together with the apical part of the follicle between the openings of the ducts and the surface of the skin with which it is in continuity, are formed at the beginning of the animal's life and persist; at the basal end the arrector muscle is attached. The bulb and the outer and inner root sheaths are developed in the early part of each anagen phase, and in the catagen phase disappear or are transformed into new structures. The papilla, which may be regarded as an organ invading the follicle, is formed early in each anagen phase, and in the catagen phase comes to have a rather different shape inside the basal saccule and retains this shape through the telogen phase, the basal saccule being disintegrated at the beginning of the next anagen phase. The hair itself is produced

in greater part during the anagen phase, completes its growth in the catagen phase, remains in the skin for a varying length of time, and is then lost. A club-sheath is formed in each catagen phase, and lasts as long as its hair remains in the follicle. The hair germ usually appears late in the catagen phase, remains dormant through the telogen phase, and then by growing begins again the cycle in which all the transitory parts, including another hair germ, are produced afresh. The use of the term "hair generation" in the way it has been employed therefore seems fitting.

Residual pigment. The formation of residual pigment has been mentioned as a feature of the catagen phase in many follicles. Frequently the hair germ is touching the pigment mass which is flattened at the surface of contact. At a slightly earlier stage the pigment may be arranged in a less regular manner, but by the end of the catagen phase the mass has generally the form of a half sphere (Figs. 21 and 22). The distribution over the body of these pigment masses is in general the same in all chocolate animals in the telogen phase of each of the first three hair generations. They occur throughout one large area on the dorsal surface and some way down the sides, but not mid-ventrally. Near the margin of this area the average amount of pigment in the basal saccules becomes less. The extent of this area is not quite the same in all animals. Sometimes the eyes are within this area, sometimes not, and it may or may not reach to the areoles of the snout and tail. The only residual pigment found in an areole was in the basal saccules of a few hairs on the snout, but most of the areoles lie outside the area that has been defined. The distribution over the skin is very regular (Fig. 22). The explanation is that the great majority of the overhairs, though not quite all, have pigment in the basal saccule, whereas it occurs in but very few of the underfur hairs. The orderliness in the distribution of the hair types is indicated in Fig. 10. In the saccules of hairs of both groups the quantity of pigment may be smaller than is usual for the part of the skin from which they come, sometimes a mere trace.

More details are wanted as to the precise part of the catagen phase in which the pigment under consideration is formed, and the manner of its enclosure in the saccule. It is sufficiently clear from a series of preparations that very often when there will be pigment in the saccule the root contains pigment throughout the catagen phase. On the other hand, it sometimes seems likely that after the whole of the pigment to be incorporated in the hair has passed upwards, there is a short interval before more pigment is formed. Once the basal saccule is fully formed it seems unlikely that more pigment is produced there,

The disappearance of the basal saccule when the telogen phase is brought to a close by the growth of the hair germ has already been recorded. Observations on the time of appearance of the different generations of hairs have shown that even if a telogen phase be unusually prolonged the saccules persist intact until the succeeding anagen phase begins. In dissection the saccules are easily detached from the dormant germ, but it is clear that in most cases they are actually ruptured when the germ increases in size. The ultimate fate of the remnants has not been determined; by the time the new hair tips are just protruding above the surface of the skin I have not been able to see anything of the scattered pigment.

With some frequency pigment is found in unusual amount or position in the basal parts of a hair, or it may be present in parts of the follicle where it does not commonly occur. In the numerous cases observed the basal saccule below such a hair contains at least the ordinary amount of pigment. Whether owing to excess or to delay in the production of pigment, it would seem that more than the ordinary amount has been present in the catagen phase, and that this has found its way into places normally free from pigment. Two examples of swellings containing such pigment in the basal parts of hairs are figured (Figs. 26 and 27). In the first case there were also small scattered masses in the hair germ. Occasionally pigment is lying against the hair-club, or perhaps a little pigment may be actually inside the thin tissue of the club-sheath itself. In such cases the pigment is obviously not affected by the rupturing of the basal saccule, but remains where it is when a new hair is grown, so that from time to time one comes across follicles containing one hair that affords an example of what has just been described, and a hair of the next generation with ordinary residual pigment in the saccule.

In the large vibrissae of the snout what is regarded as an abnormality in the hair-clubs described above is the rule. The clubs of the longer vibrissae contain pigment (Fig. 28), but the quantity is small, and the shape of the club is the same as in vibrissae that do not contain these little pigment masses. Now as soon as one of the longer vibrissae has completed its growth the development of a companion starts in the same follicle, and from the examination of a series of follicles it is concluded that at no time in the passage from the catagen phase to the anagen phase, which so quickly succeeds it, is the bulb without pigment.

Residual pigment has been found in coloured mice of all the types in which a search for it has been made. These are as follows: Wild, captured on a farm far from the scene of any breeding work with mice;

Black Agouti heterozygous for both Black and Agouti; Black; CC Chocolate; Cc Chocolate; Blue, or dark-eyed dilute Black; Champagne, or pink-eyed dilute Chocolate; and Yellow. In the Wild Rabbit I have found pigment in basal saccules like those of the Mouse in shape, and in their relation to the other basal parts of the follicle. It would seem from a passage by Löbner (p. 87 in work cited) that, in mammals where the papilla is to serve again, he was familiar with pigmental accumulations in the epithelial cone between the club-sheath and the papilla.

Time relations in stage of development in neighbouring follicles on the main area. It is possible to define the order in the different parts of the main area in which the anagen phase of any particular generation begins, and that in which the telogen phase is reached. In passing from one point to another in the skin between which there is plainly a difference in developmental stage attained, the transition may be gradual or rapid, while occasionally adjacent follicles show widely different stages. At the same time the changes from phase to phase in any skin are not very numerous, and there is co-ordination between the stage of development in neighbouring follicles so that it is possible to plot a not very large number of areas showing the anagen, catagen, or telogen phase of one generation or another; the boundaries of these areas are as a rule sharply defined. The history of the growth of the first three hair generations that has been worked out from a series of such charts will be related in another section. It may be stated that in normally grown animals the hairs of the first generation (G_1) have all completed their growth at something like one month, those of G_2 usually at two months, and those of G_3 sometimes at three months, though often not until later; ordinarily the growth of G_4 is in progress on the lower part of the body before G_3 has finished growing on the dorsal regions. The present observations, which are concerned chiefly with the relation between neighbouring follicles in the time of passing from one phase to another, are preliminary to the later account. It will be convenient to indicate the hair generation to which a phase belongs by a Roman numeral, and to define a boundary by naming the two phases, putting the earlier one first.

The boundaries Anagen I-Catagen I and Catagen I-Telogen I are as a rule not so sharp as those between later phases. This is no doubt because the initiation of growth in the follicles is spread over a week. There is often a strip of appreciable width where roots in which medulla is still being produced and roots in the early catagen phase are both present in considerable numbers. Also there is often a similar intermingling of follicles in the late catagen phase and those with club-hairs.

The largest overhairs (Types A and A-B) are prominent amongst the last hairs to complete growth, as also are short, slim zigzags. The former start their growth early, but are the biggest ordinary hairs of the main area; the latter I believe to be some of the last hairs to begin to grow. Boundaries that do not stand out clearly are represented in the charts (pp. 338-340) by a dotted line.

With generations later than the first, intermingling of follicles in different phases in anything like equal numbers usually occurs within very narrow limits, if at all; such follicles as there may be in the next later phase within an anagen or catagen area are as a rule situated quite close to the boundary. A phase begins at much the same time in all the follicles on the same boundary, and where any differences are in evidence I cannot generalise about any relation with hair type. In spite of what has just been said, the Anagen II boundary as it advances over the Telogen I area is often not very easy to define. This is because well inside the region in which one is satisfied that growth has started there has been only a little growth in the hair germ, so that it may be difficult to locate the actual margin. Later on the boundaries are nearly always well defined, sometimes with diagrammatic sharpness that surprises one in peeling off the tissues underlying the hair roots when preparing the skin for examination. Fig. 29 shows the boundary of a new anagen area within which there is a rather gradual increase in length in the roots. Fig. 30 shows the boundary of another anagen area within which the roots become long in a very short distance, the shortest of all containing a cone of pigment that is easy to see. This is a very common feature of the boundaries of later anagen areas, and must surely indicate that the early stages of development are passed through more quickly than in the first two generations. At the same time I do not think that what one sees at these boundaries in the preserved material used gives a true picture of what happens in the living mouse. Where a special search has been made at and close to the boundary, I have failed to find any stage intermediate between, on the one hand, an undeveloped hair germ, and on the other, a follicle with the apex of the hair tip near the surface of the skin, and (large overhairs excepted) with the medulla in course of formation. Besides those of the telogen area I have found a few undeveloped hair germs just within the anagen area itself, amongst the young hairs that have all developed to the extent just indicated. The animals were usually narcotised or killed by chloroform or coal gas, dropped into absolute alcohol, and skinned a day or two later. Sometimes they were killed by chloroform or gas, skinned at once, and the

skins fixed in alcohol or sublimate. It would seem likely that growth may continue in all follicles, where it has actually started, for an appreciable time after the initiation of the anagen phase in any follicle has been prevented.

Here and there, as some of the charts show, the early anagen or late catagen phase will be found in numerous little patches, the number of such areas drawn being necessarily only approximate. It is at the first beginning of an anagen phase that this appearance is most frequent, the small areas later joining one another. Afterwards an anagen area spreads for the most part by growth beginning in follicles adjacent to the boundary, and not so much by fusion with small patches initiated at a little distance. Fig. 31 shows the passage from late Anagen III through Catagen III to Telogen III. The catagen area is distinctly narrow, as it often is with that hair generation. Occasionally hairs in the catagen phase are so few that it is not practicable to show a catagen strip on the chart between the anagen and telogen areas (Chart 9). At the same time cases do occur, especially on the head in the older animals of the series, where there really are no follicles in the catagen phase at an Anagen-Telogen boundary. On the head, too, telogen areas of two consecutive generations may border one another (Charts 10 and 12). A Telogen I-Telogen II boundary is easy to recognise, but in later cases of this kind it may hardly be possible to trace the actual boundary, owing to the falling out of some hairs from the skin, and such boundaries have to be drawn as dotted lines.

2. *Retention of Hairs.*

A club-hair is hardly the object to remain in the skin indefinitely. In some mammals the shedding of hairs takes place periodically, in others it is not confined to any particular period. It is evident from the literature, especially from de Meijere's work, that the falling out of one hair may or may not be brought about through the presence of a new hair in the same follicle.

In the Mouse the largest number of club-hairs that I have found in the same follicle is four, and whatever the age of the animal I have not succeeded in finding a growing hair in a bundle containing more than three club-hairs. In the skins used for studying the later stages in the order of appearance of the hairs of G_3 , which were of ages up to four months, wherever there were other grounds for believing that the Telogen IV phase had been reached on some part of the venter, four club-hairs were always found in some bundle. In all such cases the four

hairs have been zigzags, and this is true for bundles of four from older animals. Yet even where every hair is a zigzag most of the bundles contain either two or three hairs; only a small proportion have four. If we consider animals up to four months in age and leave special hairs out of account, repeated dissection in anagen and catagen areas suggests that, within any area where growth has well started, one new hair is growing in each follicle. Consequently in any area in any particular telogen phase every follicle will have given rise to the same number of hairs. It follows therefore that many hairs have fallen out.

On telogen areas on both the dorsum and venter of older animals a few bundles of four have sometimes been found. By the time G_3 has finished growing the animal is not far from full grown, and though the matter remains to be investigated thoroughly there is reason to believe that after the age of 4 months a follicle does not give rise to new hairs so frequently.

It will now be plain how the order of appearance in the various parts of the body of the first three hair generations has been worked out. The distribution of the different growth phases, with a record of the largest number of hairs in a follicle in each area, was marked on charts for a series of animals of known age. It may be added that the dissection of little pieces of skin that included one or more boundaries between areas in different developmental phases was often specially useful. It will further be clear, bearing in mind that there is a good deal of variation in the dates for the third generation, that this method would not by itself be satisfactory for work on the order of appearance of later generations.

The shedding of hairs does not occur to any extent before the Telogen II phase, and then takes place gradually. There are differences between types in their retention in the skin. The following statement is based upon work on the association of hair types in the same follicle, supported by observations made in studying the order of appearance of the hair generations. Monotrichs fall out before their successors, which are also monotrichs, become club-hairs.* Type A-B hairs may stay in the skin after one companion, also a large overhair, has become a club-hair, but they appear never to remain long enough to have two companions. Awls and auchenes may be retained a sufficient time for two more club-hairs, generally also small overhairs, to be added to the bundle, but on present evidence not longer than that. Auchenes are retained better than awls. Zigzags, as already indicated, remain in the skin the longest.

The statement that two fully formed monotrichs have never been

found in the same follicle in any mouse is made after special searches on the mid-dorsum and mid-venter and much dissection on other parts. On a Telogen II area on the mid-dorsum some of the longer-tipped Type A-B hairs of G_1 have club-hair companions, others do not. Monotrichs of G_2 and G_3 tend to be longer and broader than those of G_1 , and it will be understood that in those generations some hairs, which from the consideration of total length, tip length, thickness of cortex, and shape of cross-section, are classed as Type A, are III-septulate. Such hairs have always been found to be without companion club-hairs, but the shorter of the large overhairs, approaching Type B in structure, often have them. Monotrichs are known to have successors of their own type because the two hairs can be found together when the younger hair is in the earlier stages of the anagen phase. The older hair is often still there when the newer has made two-thirds of its growth in length, but on present observations not when the later hair has reached the catagen phase. This fact alone suggests that monotrichs fall out, not because they are fitted to remain in the skin for only a short time, but because they are forced to go. Further evidence is provided by the poorly grown animal No. 60 *b* (see p. 313). G_1 completed its growth in this mouse in average time, reaching the telogen phase on the sacrum in 19 days. After that there had been no further growth of hairs on the dorsum when the skin was preserved, ten days later than G_2 normally finishes growing. Yet monotrichs were still there in the same sort of numbers as when G_1 is growing. On the general surface of the body in other animals de Meijere often found isolated stouter hairs surrounded by bundles of finer hairs, and Toldt's Leithaare are alone in the follicle. To some of these cases the present explanation would very likely apply.

It will be shown later that there are great differences in retention between the various special hairs, but it may be mentioned here that two vibrissae have never been found together as club-hairs in the Mouse. The full-grown vibrissae found in the skin at the same time vary very much in length and the larger take longer to grow than the smaller, but it is believed that as soon as any vibrissa is completely formed its successor begins to grow. Here also one may suppose that as the later hair is finishing its growth the earlier one is in some way forced out of the skin. The same thing may well happen with the vibrissae of other animals, for Maurer (quoted by Hofer) says, "Einzeln stehende Haare kommen allen Säugetieren zu, so die Sinushaare." In terms of function the significance of the fact that full-grown monotrichs and vibrissae stand isolated in the skin may well lie in the former, as well as the latter, being

tactile hairs. Were two such club-hairs to be retained in the same follicle confusion might be expected in the transmission of stimuli.

Hairs of other types do not seem to be forced out of the follicle unless it be some of the Type A-B hairs approaching Type A in structure, though possibly later hairs in growing loosen earlier ones, especially when the distal ends are broad. The long retention of the zigzags may be accounted for in two other ways. The base is slim and the weight of the hair comparatively small, and protection is afforded by the awls and auchenes. Very likely the waviness of the zigzags hinders the movement of air in the coat and so helps in conserving the body heat, but one also wonders whether the jar produced at the base of the hair by contact with solid objects may not be reduced by the series of constrictions at which the shaft bends easily.

The only observations upon retention on different parts of the body were made on a female rather more than a year old that had suckled a family up to her death. There seemed to be rather more bundles of four hairs on the mid-dorsum than on the mid-venter, though very few were to be seen on either part, but bundles of three were much more plentiful on the mid-dorsum.

3. *The Order of Appearance of the Hairs of Generations I, II and III on the Main Area.*

The important facts about the history of hairs on the main area that lead up to the subject of this section may be summarised as follows:

1. All hair-bundles are believed to be founded in the early days of life.
2. Many hairs are retained in the skin for a considerable time.
3. At the growth of each hair generation within any area it is believed that one hair, and one hair only, is produced in every follicle.
4. Apart from occasional exceptions in small parts of the skin, after the completion of the growth of a hair of any generation there is an interval of time before the formation of the hair of the next generation begins in the follicle.

5. Hairs in the same stage of growth have simple relations to each other and to hairs in other stages in respect of their distribution on the skin. With these spatial relations time relations are necessarily bound up.

The space and time relations, as studied in entire skins, may now be considered.

The normal order of appearance of the first three hair generations is illustrated by representative charts numbered 1 to 11¹. The few skins

¹ See pp. 338-340.

in which the major features are unusual will all be described; such departures from the normal lend themselves to very simple explanation, for the animals were poorly grown or unhealthy. Chart 12 is an example. None of the rest examined failed to agree on important points with the sequence shown in the other charts, but minor differences in comparable skins will be described.

Further data about the order of appearance of the first three generations were obtained by pulling out hairs every day, or every few days, from the mid-venter, sacrum, and vertex (G_1 and G_2 only) of a few living mice. The hairs do not often break, and in the growing stages the long roots come away sufficiently often. It is a disadvantage that the hairs do not hold together in bundles, so that each time one is taking hairs from a slightly different place, but the results support those obtained in the study of preserved skins, in particular more being learnt about the time required for the different phases. Thus it was found that the catagen phase in any follicle must last from one to two days. The skins of two of the animals used in this work will be specially described, as they show unusual features. Both in the tufts pulled out, and afterwards in the preserved skins of some of the same mice a look-out was kept for departures from the normal in the structure or manner of growth of hairs. All that was found was about a score of very abnormal hairs, amongst those pulled out from four of the animals, believed to be the products of follicles from which a growing hair had been torn out.

Generation I. The early history of the first generation has been described under "Hair Development." The first hairs to complete their growth are those at the front of the head at the margin of the snout areole, on a little ring round the root of the tail, on a little band laterad and posterior to the ear where the hairs are specially short, and on the fore- and hind-legs on the small area omitted from the charts. This is at about 15 days. The telogen area spreads backwards from the front of the head, and in two or three days the whole of the head is in this phase, except for a little area just mediad to the ear with specially long hairs where it is not reached for another day or two. At about 17 days, too, growth is first completed in hairs on the abdomen, in the mid-ventral line. The telogen phase is reached a little earlier on the vertex than on the mid-venter. The telogen areas of the head and abdomen increase in size and unite. The little area at the root of the tail gets very little bigger, if any, but is in due course joined by an extension from the club-area of the abdomen. The last hairs to complete their growth are on the sacrum, at about 19 days. This is the average time on the available data, but in some of the charts

older skins are figured with the growth of this generation still not completed.

Generation II. See Collins, 1923, pp. 76 and 78, for *Mus musculus*, as well as his descriptions of moults in species and races of *Peromyscus*, and his general discussion of moults in mammals.

The Anagen II phase was always first observed on the venter near the fore-legs. G_2 usually begins to develop just before G_1 has finished growing on the sacrum, and at something like 18 days. Often it is later, and sometimes the growth of G_1 is already complete. The anagen area soon extends all over the ventral surface, quickly reaches the ears and eyes, and spreads on the dorsum. Commonly growth starts on the sacrum rather later than on the anterior part of the back, on the vertex later still, and last of all on the root of the tail, and on the parts of the legs not included in the charts. Occasionally, too, growth begins specially late on the front of the head, dorsally, just behind the snout areole. The age at which the anagen area completely covers the sacrum is estimated at 28 days, the head two days later, and the root of the tail and legs several days later still.

The Telogen II phase was seen first on the under surface of the head in the middle line, at 33 days. The telogen area quickly enlarges on the lower surface of the body, of which the last part to show club-hairs is close to the hind-legs. The data from all sources point to 36 days as the time of completion of growth on the mid-venter. On the dorsal surface growth is finished a little earlier on the anterior part of the back than on the sacrum (about 46 days), and on the vertex the time is still later, but only about one day. At the anterior end of the dorsal surface of the head club-hairs are sometimes found very early, but sometimes not until late. Observations close to the base of the ear, both laterad and mediad, mostly agree with those made for G_1 . On the root of the tail I have no observations to show that the telogen phase is reached there later than on the adjoining part of the back where the anagen phase appeared much sooner. On the limbs in the parts left out of the charts it is late before growth is completed; even within those small areas there are considerable differences in the dates of the several phases. Fig. 29 shows the Telogen I-Anagen II boundary from the fore-leg of No. 49 *a*. There is sometimes a Telogen I-Telogen II boundary at the base of the legs. Perhaps more often than not the first G_3 hairs are growing on the abdomen before the development of the last of G_2 has started on the legs.

Generation III. The hairs of G_3 start to grow first on the ventral surface, at about 45 days. In several mice growth was found to be start-

ing in the neighbourhood of the mammae, or in the corresponding region in the male, sooner than along the mid-ventral line; growth is also rather late in beginning close to the legs. Dorsally the anagen area quickly reaches the ears and eyes. It is perhaps a little later in covering the sacrum (about 55 days) than the anterior part of the back, but on the dorsal surface of the head there is great delay. Individuals differ greatly in this respect, but the average age when all the hairs of G_3 have started to grow on the head may be put at 75 to 85 days at least. There is also delay at the root of the tail and on the legs.

G_3 club-hairs are found first just anterior to the front legs, at something like 60 days. The telogen area soon covers the ventral surface, the part near the hind-legs, as in G_2 , being the last to be included. The sacrum (somewhere about 75 days) is a little behind the anterior part of the back, and the root of the tail often still later. The dorsal surface of the head is very late, the growth of the whole generation coming to an end there at anything from 90 to 100 days or more. Within the head area differences in detail have been observed. In one or two cases it has been possible to observe that growth was late in starting close to the ear on its mediad side. On the fore- and hind-legs an account similar to that for G_2 could be given. G_4 hairs usually begin to grow on the abdomen before the latest G_3 hairs on the legs, and G_3 hairs complete their growth on the legs a good deal earlier than on the vertex.

In all the skins of my series showing the late stages in the appearance of G_3 the fourth generation had begun its growth. It starts on the ventral surface on each side, not on the mid-ventral line. In many animals there were little Telogen IV areas at the anterior end of the body. It may be added that where hairs which there is good reason to believe to belong to G_4 have been found growing on the back, they are in patches, and do not show the simple regularity in order of appearance of the earlier generations. The same is true of the growing hairs found anywhere in the skin of the small number of older animals examined of various ages up to a year and a half.

Minor differences between comparable skins. Various differences in detail in the order of appearance can be seen even in the charts given. These are most marked near areoles, in little corners, one may say, of the main area. The two extremities, anterior and posterior, are notable examples. At the front of the head, on the dorsal surface just behind the snout areole, both G_2 and G_3 may start their growth either early or late, sometimes very late. Other differences are in evidence on the vertex where, certainly, the long time over which the beginning of the growth

of hairs is spread makes it easy to detect small variations. At the root of the tail G_3 starts a good deal later, both relatively and absolutely, in some animals than in others. On the limbs the charts indicate that there is sometimes a boundary between the telogen areas of two successive generations and that after an interval growth begins again on the side of the boundary with one generation of hairs the fewer; in other cases there is no such break.

Unusual features in the order of appearance in certain animals. Some differences in the age at which the same general stage in the order of appearance is reached will be noticed in the charts, and further examples could be given. The four cases now to be described show much greater delay for the particular age, taking the skin as a whole, and in two instances the delay is greater in some parts than in others.

No. 60 *b* grew well enough for the first three or four weeks but after that never seemed to get any bigger. G_1 was completed in normal time, but after that the hairs pulled out never included any in a growing phase. The animal was killed. The only G_2 hairs to be seen are a few small patches and isolated hairs in the anagen phase near the fore-legs and in the middle of the venter. G_2 , it appears, is starting in the ordinary manner, but some five weeks late.

No. 83 *a* was small for its age when it died. G_2 is everywhere complete and there are patchy Anagen III areas in the region of the mammae. G_3 is beginning in the usual way, but a month late.

No. 60 *a* (Chart 12) was small for its age when killed. A continuous Telogen II area covers most of the body, but there are Telogen I areas on the vertex, the root of the tail, and the limbs. Several Telogen I-Telogen II boundaries will be noticed. The delay on the head reminds one of what normally happens in G_3 . The condition revealed in this skin may be described in terms of the rates at which two things happen. Consider, firstly, two positions between which there is normally a certain interval of time in the starting of growth of G_2 . In this animal this interval has clearly been longer than usual. If we think of the point at which growth begins as moving along the skin from one position to the other, we may say that the rate of movement of the point has been less than normal. Secondly, we have to think of the rate at which a hair itself grows, once its formation has actually started. This rate may, or may not, have been less than normal, but even if it has, any proportionate decrease in the rate of growth of the hairs themselves is small compared with the decrease in rate of movement along the skin of the point at which the growth of the hairs is just beginning.

No. 77 *a* grew well enough for most of its life, but died after looking poorly for a week. The history of its hair generations as learnt by pulling out hairs was as follows:

	Mid-venter	Sacrum	Vertex
G_1 , growth completed	17 days	19 days	16 days
G_2 , growth completed	36 days	43 days	43 days
G_3	Growth completed, 67 days	Anagen phase never found	—

After death the anagen phase was nowhere evident on the main area, club-hairs alone being found. Right along the mid-ventral line bundles of three hairs were found, but all along the mid-dorsal line only two hairs in a bundle. It was ascertained that between the mid-venter and the mid-dorsum the Telogen III phase extended from the middle line on the abdomen up to about a quarter of the distance between the two positions. The situation may be described in this way. The rate of movement of the point at which the growth of hairs begins has fallen to zero, but all the hairs that started to grow have kept growing and become club-hairs. Here is brought out even more strikingly than in No 60 *a* the distinction between the beginning to grow on the one hand and the growing on the other. In this connection what has been said about the margins of Anagen III and Anagen IV areas may be recalled (p. 312).

On the areoles of the four skins just described there is some evidence of delay in the beginning of the formation of hairs, but it is less marked than the delay on the main area.

* *Discussion of data presented.* In contrasting the development of G_1 on the one hand and G_2 and G_3 on the other, attention may be drawn to the greater uniformity in the first commencement of hair growth throughout the main area in G_1 , to differences in the parts which are in advance of others, and to the close agreement in the time when neighbouring hairs, whatever their type, begin to grow in the later generations. One notices that the parts of the body where G_2 and G_3 are specially late in starting to grow, namely the head, the root of the tail, and the legs, are largely composed of bone, which lies close to the skin.

The time at which the growth of a hair generation is completed in a given place depends, naturally, both on the time when growth starts and on the period occupied by growth. To take the second factor first, its effects are most clearly seen in G_1 , and easily too in G_2 . It is evident that the time required for complete growth in any region bears some relation to the length of the hairs there. The marked differences in the date of the end of growth in G_2 and in G_3 in parts of the body arise

chiefly from differences in the date of the beginning of growth in the various regions. In badly grown animals some account has just been given of the effects of late commencement of growth.

In G_2 and G_3 it is easy to think of the growth of the hairs as started by a wave passing over the body in a simple easily defined course. At the very beginning of growth it is as if a number of little waves joined forces to make a single large one. Sometimes this wave runs an uninterrupted course over the whole of the main area, splitting towards the end into several sections, and often flowing round a barrier that one can imagine to exist on each side of the head. Sometimes, in certain small areas, the process is completed by subsidiary waves that are initiated after the original wave has come to a stop.

In both G_2 and G_3 the wave runs the same course. The G_2 wave starts at about 18 days after birth, or probably 20 days after G_1 began to grow; the wave begins usually just before G_1 has quite finished growing. The G_3 wave starts at about 45 days, or something like 27 days after the G_2 wave began. This wave, like its predecessor, generally starts at about the close of the growth of the previous hair generation, though what has been said about the late start of G_2 on the legs may be recalled. The G_4 wave commonly starts well before G_3 has finished growing, and nearly always, too, before the G_3 wave has run its course. From start to finish, apart from the frequent delay on the legs, the G_2 wave is estimated to take about 12 days, ending on the vertex one or two days later than on the sacrum. The G_3 wave takes something like 35 days, but as far as the sacrum the time occupied in this generation, as in the previous one, is put at about 10 days. In G_3 delay on the head is a normal thing; in an animal small for its age there was also delay in G_2 . One does not know whether the explanation is to be looked for in some factor, such as the presence of bone near the skin, that is local to the head, or whether this region, being the last part of an established track, is merely showing the effect of a reduction, however brought about, in the rate of travel of the wave.

The work of Collins and his review of literature shows that the wave phenomenon is found in other mammals. Leaving out of account the time relations between follicles, it is evident from the work of de Meijere that the other features in hair succession described in the Mouse are common in mammals. Given those other features, and they are simple ones, the time relations of the wave phenomenon are of the simplest kind one can imagine, unless growth should start simultaneously throughout the main area.

4. *Special Hairs and their Modes of Succession.*

The hairs of areoles. The vibrissae and hairlets are discussed in later paragraphs. The special hairs near the mammae are dealt with here.

Save that the hairs of the areoles of both feet are much alike, each areole has its characteristic assemblage of hair forms. Examples from a few of the areoles are figured (Figs. 34 to 37). The spindle-shaped hairs of the tail are perhaps the best example of a kind of hair peculiar to a particular areole. On the same areole there is often a big change in the general size and form of the hairs in quite a short distance, as on the convex surface of the ear at points progressively further from the base. In some cases hairs growing side by side differ greatly in size, as again shown on the ear (Fig. 36), but on the tail there is little difference between neighbouring hairs (Fig. 37). Most hairs on areoles, including the smallest on the ear, are built on the overhair rather than the underfur plan, but the special hairs near the mammae are more like zigzags. On each areole there are hairs that can easily be distinguished from those of the adjacent part of the main area, but where the hairs of the two regions approach one another in form distinguishing structural features cannot be defined. This resemblance in form is found, of course, more especially near the boundary between the two regions. In consequence of the difference in mode of hair succession the boundary is usually not difficult to define when one examines several animals showing different developmental phases on the part of the main area bordering an areole. In most cases the boundary is quite sharp, as one may see well on the tail. In other cases there is a mingling together of follicles that show the two modes of succession, as on the scrotum especially, and to a small extent at the base of the ear, while the special hairs near the mammae might all be regarded as growing amongst the ordinary hairs.

As on the main area, I believe that follicles are formed only when the animal is very young. Nearly all begin their development in the 1st week, but at the growing margin of the ear the time is a little later, and at the tip of the lengthening tail some are laid down at least as late as two weeks. The chief matters to which attention has been paid on the areoles are the retention and succession of hairs as revealed by dissection in a series of skins. Retention is longest on the ear, fairly long on the tail, sub-caudal areole, part of the snout, and part of the feet areoles, short on the snout where the hairs are shortest, round the eye margin, and near the claws. The special hairs round the mammae are very few in number but appear to be retained fairly well. The longer the retention

the smaller is the average number of growing hairs found at one time after the original hairs have completed their growth. Thus with the short hairs on the snout the process of renewal begins as early as 7 days, and growing hairs are found there at all times. On the ear the first new hairs were observed at 7 weeks and even at 4 months in some follicles there has probably been no fresh growth. Growing successional hairs appear to have a haphazard distribution even within a small part of an areole, and neighbouring follicles show different developmental stages. Even so, growth sometimes does begin at a definite part of an areole and gradually extends on to the rest. The best example of this is afforded by the tail round the age of 5 weeks, development being progressively later towards the posterior end. A certain approach to the mode of succession of the main area is thus found sometimes.

Vibrissae. This account has reference to the vibrissae situated on and near the snout areole, and not to the few carpal vibrissae. Failing a knowledge of histological detail in the follicles of the various hairs of the snout areole, the term vibrissa will be applied to the large, stiff hairs, as much as 3 cm. long, that one could call whiskers at sight, and to smaller hairs, down to a third of a centimetre in length, which resemble them in general shape and in the pattern of the scale margins, these shortest hairs being without medulla. There are also much shorter hairs that resemble in certain features the undoubtedly tactile long hairs, and they may well also have the same function, but they are not taken into consideration here. The vibrissae are characterised by a long apical region without medulla, by an approximately circular cross-section, by having the region of greatest diameter only a short distance from the base, by the shape of the club (Figs. 28 and 34), and by the form of the scale edge pattern (Figs. 118 to 120). Something has been said earlier about the development of vibrissae and about the presence of pigment in the club of the larger hairs and its presence in the follicle throughout the period covering the completion of the growth of one hair and the commencement of growth of its successor. It has already been recorded that the succeeding hair begins to be formed very soon indeed after its predecessor has become a club-hair, and that the earlier hair goes as the later one is nearing completion, being probably in some way forced to fall out. Not only is the companion hair of a vibrissa always a vibrissa, but there is reason to believe that all those grown in the same follicle are of something like the same size. Outside the snout areole the following vibrissae are recognised on each side of the head: two supra-orbitals close to the anterior margin of the eye; one sub-orbital a little posterior to the eye;

and either two or three a little to the side of the mid-ventral line level with the eyes. The tips of those near the eye and of some of those close to the mid-ventral line protrude at birth. By following up the hairs in the sub-orbital follicle in a series of animals it has been concluded that the first club-hair is fully formed in the 4th week, the second at 7 or 8 weeks, the third at 10 or 11 weeks, and the fourth in the 4th month, so that at 4 months it is probably the fifth hair which is growing. The earliest sub-orbital vibrissa measures about 1.3 cm. in length, those found as club-hairs after 3 months about 1.5 cm. On the snout vibrissae about $\frac{1}{3}$ cm. long are club-hairs at 2 weeks, and the larger ones complete their growth in turn according to length. In the 5th or 6th week the longest of the original vibrissae finish growing. From the examination of the series of skins the growing companions of the longest vibrissae in an animal of 4 months are regarded as the fourth hairs in the succession. In the same time half a dozen or more club-hairs will have been produced in the follicles containing short vibrissae.

Hairlets. Hairlets (Figs. 32 to 34) are very short hairs without medulla with a characteristic slim appearance, being thin in comparison with other small hairs of similar lengths that may also not have medulla. The shortest hairlet found was .026 cm. long and 4μ in greatest diameter. Except when situated amongst other special hairs hairlets are rarely longer than .06 cm. and 7μ is the greatest diameter recorded. The cross-section is practically circular. The pattern of the scale edges is annulate, with longitudinal margins connecting adjacent rings scarce; one short spiral scale edge is figured (Fig. 121). A transition in form has been traced from hairlets to other hairs on the snout, the ear, and near the mammae.

Hairlets have been met with practically everywhere on the main area, and on the snout, ear, eye-margin, and sub-caudal areoles, and close to the mammae. On the snout they are mostly grouped close to vibrissae (Fig. 34), a vibrissa having a ring of as many as ten or more. The vibrissae on the main area on the head and the fore-leg are also surrounded by them. Hairlets are quite numerous near the ears, but elsewhere on the main area they are usually only found close to monotrichs (Fig. 32), but by no means all the monotrichs are attended by them. Usually there is only a single hairlet close to a monotrich, but now and then two, three, four, and even five have been noted.

The development and succession of hairlets has been followed on the snout and close to the ear. In both regions they have been recognised with certainty at 9 days, but development must start several days

earlier. At 13 days some have become club-hairs, and all at 16 days. Growing hairlets are found very rarely indeed after the original ones have become full grown, the vast majority being isolated club-hairs. It is impossible to generalise about the dates when new hairlets grow. No. 36 *a* is the youngest animal in which two club-hairs have been found in the same follicle. Three full-grown hairlets were found in the same follicle near a mamma of No. 84 *a* (Fig. 33).

In some dozen cases a hairlet has been observed in the same follicle as one or more hairs of another kind, either special hairs on areoles or zigzags on the main area. Just once a hairlet has been found in company with a zigzag higher in the follicle than itself, and so presumably older.

5. *Hair-type Associations within Single Follicles.*

When more than one of the special hairs dealt with in the last section are present in a follicle in most cases all are essentially alike. This is very striking when an areole bears hairs of different sorts side by side. A few exceptions have been mentioned in the account of the hairlets. On the main area one soon notices that all the hairs in the same follicle may belong to the overhair or to the underfur, or that both groups may be represented. In other animals de Meijere has shown that different kinds of hair often occur in the same bundle.

The relative age of the several hairs in the same follicle is easy to determine. If a hair is in a growing phase it is obviously the newest hair present. If all the hairs have completed their growth G_1 , G_2 , and G_3 are distinguished in the way explained under "Hair Development" (see Fig. 20). It may be added that a G_1 hair will often differ in structural features from companion hairs of G_2 and G_3 . Hairs of those later generations have not yet been studied in much detail, but they are very often longer and stouter than hairs of the first pelage. A later overhair generally contains more inside septules, and maintains a considerable breadth more proximally; a later zigzag, apart from the almost invariably greater length, usually has a proportionately longer second segment (Fig. 42).

Type-associations in the first two hair generations. Hair types in alphabetical order from A to D are spoken of as successively "lower," or in the reverse order successively "higher." Some five thousand pairs of hairs from nine chocolate animals were in all examined from the mid-dorsum and mid-venter. The comparable data for the two regions are given in Table II. A G_1 hair may have a G_2 companion of the same type, quite often of a higher type, but only rarely of a lower type. Where the G_2 hair was not of Type D the G_1 hair was more often than not of

TABLE II.

 G_1G_2 Hair-type associations.

Comparable data from mid-dorsum and mid-venter.

The figures are totals composed of counts from 500 follicles from each region from four animals.

The percentages of G_1 hairs which had fallen out from these skins were small.

Type-association		Mid-dorsum	Mid-venter
G_1	G_2		
A	A	32	19
A-B	A-B	17	2
B	A-B	12	4
B-C	A-B	2	—
C	A-B	1	—
B	B	70	111
B-C	B	1	—
C	B	137	85
C	B-C	1	—
C	C	55	2
C-D	B	2	1
C-D	C	8	2
D	B	2	74
D	B-C	1	2
D	C	174	182
D	C-D	21	23
D	D	1453	1493
B	D	2	—
B	C	2	—
C	C-D	2	—
C	D	4	—
C-D	D	1	—
Totals		2000	2000

Totals of each hair type in above data.

Hair type	Mid-dorsum		Mid-venter	
	G_1	G_2	G_1	G_2
A	32	32	19	19
A-B	17	32	2	6
B	86	212	115	271
B-C	3	2	—	2
C	200	239	87	186
C-D	11	23	3	23
D	1651	1460	1774	1493
Totals	2000	2000	2000	2000

It will be understood that a G_2 A standing alone is counted as a G_1 A, G_2 A association.

a lower type than its newer companion.⁶ But where the G_1 hair was of a type higher than D, and it was therefore possible for the later hair to be of a lower type than its older companion, this came about, on the available data, only once in forty times on the mid-dorsum, and never on the mid-venter. There is a limit to the degree of difference in type between hairs in the same follicle, and wide separation in type when it does occur is rare. There is a large amount of variation between individuals in the relative numbers of awls and auchenes in both hair genera-

tions. On comparing the assemblage of types on the mid-dorsum and mid-venter one finds that on the whole in the first generation the hairs grade higher on the mid-dorsum, but that in the second generation they grade about the same; this is a matter which should be followed up.

Type-associations in the first three hair generations. Small numbers of hair-bundles were examined on the mid-dorsum of three animals. As stated earlier, follicles containing hairs of types higher than B never retained more than two hairs. A-B's of G_2 and G_3 were found together, as were A's of these two generations, when the newer monotrich, of course, had not finished its growth. The following type-associations have so far been found (see Figs. 39 to 42):

G_1	B	B-C	C	C	C	D	D	D	D	D	D	D	D	D	C
G_2	B	B	B	C	C	B	C	C	C-D	D	D	D	D	C	B
G_3	B	B	B	B	C	B	B	C	C	B	C	C-D	D	D	C

The data for type-associations between G_2 and G_3 show that the hair of the later generation is of a higher type less frequently than when the association is between G_1 and G_2 . Occasionally the G_3 hair belongs to the lower type. There are indications that in the relation under consideration between G_2 and G_3 marked differences exist between individuals.

Type-associations in a few old animals. In three chocolate animals a year and a half old records were made for pairs of hairs in which the later one was recognised by the basal saccule. In all three mice pairs of hairs were easy to find in which the later hair was of the lower type. It was also observed that where the two hairs were of the same type the later one was often the shorter, or narrower, or both. When the later hair was an awl it was sometimes very like a G_1 hair of that type.

Colour in type-associations. A study has been made of the agouti colour pattern on the mid-dorsum but it is not now proposed to give an account of this work. In G_1 the largest overhairs are black throughout, the smaller have a yellow band and, broadly speaking, the smaller the hair the longer the band. In the underfur the yellow band was found in all but a few of the shortest hairs. On examining the colour relations between companion hairs of the first two generations it was found that both hairs may be black throughout, both may have the yellow band, or one hair only may have it. In the last case the hair possessing the band has almost always belonged to the first generation. In mice of mutant types, too, there must sometimes be colour differences between companion hairs, for an animal may change in colour. This change, as is well known, is very striking in mice belonging to yellow types.

SUMMARY.

1. The overhair consists of hairs named monotrichs (Type A), awls (Type B), and auchenes (Type C), the underfur of zigzags (Type D). Intermediate types, A-B, B-C, and C-D exist, but in spite of the great difference between an auchene and a zigzag Type C-D is very scarce.

2. In the light of statistical work on the hair types methods are suggested for making comparisons of the same hair generation of different individuals.

3. Features in the cuticular scale pattern are correlated with the larger structural characters of hair types. Differences in the pattern can be defined between parts of the same hair, and frequently between the ectal and ental surfaces.

4. Hair development has been studied in order to supply a basis for work on hair succession. Some attention has been paid to the small amount of pigment which is produced in some hair roots but does not find its way into the growing hair; this is called residual pigment.

5. Many hairs are retained on the main area long enough for from one to three later hairs to be grown, one by one, in the same follicle. The smaller a hair type the longer on the average is it retained.

6. On the main area the hairs are grown in distinct generations. The features of the mode of succession are summarised on p. 309. The order of appearance over the body of the hairs of generations I, II, and III is described.

7. Special hairs on anomalous areoles or in isolated follicles on the main area are described with special reference to their modes of hair succession. There are great differences in the frequency of renewal of these hairs. The mode of succession of the vibrissae is in striking contrast to that of the ordinary hairs of the main area.

8. Relations can be defined between the kinds of hairs produced in succession in the same follicle. This applies also to the colour of companion hairs in the Wild Mouse.

APPENDIX.

Methods.

Before dissection a whole skin or a part of a skin was warmed in dilute acetic acid, up to 5 per cent. in strength, for several hours. It was found by suitable tests that the number of hairs which fell out during this maceration was negligible, nor could any effect on the dimensions of hairs, examined in 70 per cent. alcohol, be detected.

Orientated hairs were measured under a cover-glass, usually in alcohol, sometimes dry, care being taken to avoid distortion from pressure. After examination most of the hairs were stored singly in tiny test-tubes.

In making permanent preparations some trouble arises from the fact that in moving hairs from one liquid to another that is denser, bubbles of vapour often appear in the hollow parts of the axial region (Nathusius, 1866, and Werneke, 1916). The use of cedar oil was given up owing to the difficulty of securing penetration. In the case of xylol, passing through a series of mixtures of alcohol and xylol sufficed when three days were allowed for the passage through half a dozen mixtures. On mounting in Canada balsam it was never possible to guarantee the result, but thin balsam usually gave mounts free from bubbles. Where clearing was not needed, or was undesirable, a dilute aqueous solution of some preservative like formalin was useful, excess liquid being removed by blotting-paper and the mount ringed with gold-size. The preparations photographed in Figs. 11 and 12 were made in this way.

Various methods designed to render the scale edges easier to see were tried, especially those given by Hofer and Hausman, but nothing was found for the particular purpose that was an improvement upon simply mounting cleaned hairs under a cover-glass dry. In orientating hairs under the prismatic binocular it often helped to moisten them with absolute alcohol or ether, and to manipulate them just before they became quite dry. Zigzags were usually cut into several pieces before being orientated for the ectal or ental surface. The cover-glass was fastened in position by two strips of gummed paper; ether could thus be allowed to run under the cover-glass, and by doing so it was sometimes possible to remove some particle of dirt, or to cause a hair which had been slightly compressed to recover its original condition. In order to examine the second surface many hairs were re-mounted. Sometimes the need for this was avoided by using a rectangular cover-glass in the place of the slide, so that these mounts, though rather flimsy, could be examined under the oil immersion lens with either surface upwards.

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DESCRIPTION OF FIGURES.

Figs. 1-52 are on Plates XXI-XXIV.

Figs. 53-122 will be found on pp. 331-337.

Hairs are from chocolate animals unless it is stated otherwise. The number of a mouse shows its age in days.

Figs. 1 to 42 are microphotographs.

HAIR TYPES.

- Fig. 1. Monotrich or Type A hair of G_1 from dorsum, broadest part of shaft. $\times 170$.
 Fig. 2. Awl or Type B hair of G_1 from mid-dorsum, broadest part of shaft. $\times 170$.
 Fig. 3. Awl of a generation later than the first from dorsum of black mouse, broadest part of shaft; inside septules numerous; within this region of the shaft there is a marked falling off in the cortical pigment proximally. $\times 170$.
 Fig. 4. Auchene or Type C hair of G_1 from mid-dorsum, constriction and adjacent parts of blade and stalk. $\times 100$.
 Fig. 5. Zigzag or Type D hair of G_1 from dorsum, distal constriction. $\times 240$.

Figs. 6 to 9 show transverse sections of hairs cut in skin from the mid-dorsum of No. 10a. The upper margin of each figure is parallel to the surface of the skin. In Figs. 6 and 7 both outer and inner root sheaths are shown, in Figs. 8 and 9 only the outer sheath, the sections having been cut nearer the surface of the skin. The inner root sheath, where present, is clear, without nuclei. Hairs of Types A, A-B, and B are cut in the broadest part. The small hairs are zigzags. The tissues but not the hairs are stained.

Fig. 6. Monotrich, nearly circular in section; cortex thick. $\times 270$.

Fig. 7. Large overhair, either monotrich or Type A-B approaching a monotrich in form; ectal surface very slightly concave, ental surface convex; cortex thick. $\times 270$.

- Fig. 8. The larger overhair Type A-B; ectal surface concave, ental convex but nearly flat in the middle; cortex about the thickness usual in Type B, but the short axis of the section is greater than in an awl. The smaller overhair section shows the form presented by an auchene cut just distal to the neck; ectal surface slightly concave. $\times 270$.
- Fig. 9. Section typical of broad part of awl; ectal surface concave, ental surface convex but except at the sides nearly flat. $\times 270$.

HAIR ARRANGEMENT.

- Fig. 10. Section of skin from mid-dorsum of No. 10 *a* parallel to surface of skin, the hairs and their sheaths thus cut in a slanting direction. Tissues stained. The hairs that do not contain constrictions are cut in their broadest part. The section is taken at a level in the skin deep enough for nuclei to be recognised in the inner root sheath, so that the contrast between inner and outer root sheaths is less than in Figs. 6 and 7. The hair types can be distinguished with not much uncertainty. The distribution of the types is illustrated, but at this level in the skin the arrangement in rows and groups is not so apparent as in other preparations. In this and the next two figures the upper and lower margins run at right angles to the mid-dorsal line, the upper margin being the posterior edge of the rectangle of skin. $\times 100$.
- Fig. 11. Skin from mid-dorsum of white mouse of 27 days, viewed from below, showing club-hairs of G_1 ; each hair alone; the hairs tend to be arranged in rows; the hair clubs are of different sizes; the sebaceous glands and their ducts stand out. $\times 65$.
- Fig. 12. Skin from mid-dorsum of white mouse of 48 days, viewed from below, showing hair-clubs, the growth of G_2 having been completed; many follicles contain two hairs, a few hairs are alone, including the largest present which is evidently a monotrich; other features as in Fig. 11. $\times 65$.

For hair arrangement in an animal of four months see Fig. 22.

DEVELOPMENT.

Anagen phase.

- Fig. 13. Early stage in development of large overhair from dorsum of new-born dark-eyed mouse; stained; the formation of the inner root sheath and the hair tip has started, but the tip does not project above the skin; no pigment. $\times 65$.
- Fig. 14. Large overhair from dorsum of No. 5 *a*; dissected away from the skin at the apical end of the inner root sheath; lightly stained; tip long, the basal end being enclosed in the follicle and containing a good deal of pigment; medulla not yet forming. $\times 65$.
- Fig. 15. Dissection from dorsum of No. 5 *a*; largest hair an awl or auchene, the others zigzags; lightly stained; in the two follicles on the right the medulla is being formed. $\times 65$.
- Fig. 16. Dissection from dorsum of wild mouse; growth of hairs of G_2 in full swing; stained; auchene in middle, zigzags left and right. $\times 65$.

Catagen phase.

- Fig. 17. Monotrich from dorsum of No. 16 *a* dissected away from the skin at apical end of inner root sheath; stained; early catagen phase. $\times 65$.
- Fig. 18. Dissection from dorsum of wild mouse; all hairs zigzags; two G_2 hairs in the late catagen phase, the other G_2 hair a club-hair; each G_2 hair has a G_1 companion. In the parts of hairs that look black throughout there are bubbles in the vacuoles. $\times 65$.
- For the very late catagen phase in Type A see Fig. 21.

Telogen phase.

- Fig. 19. Longitudinal section of hair follicle of wild mouse, the G_2 hair having completed its growth; stained; G_1 hair-club with sheath; one sebaceous gland and its duct; the duct opens at the base of the part of the follicle which persists from one hair generation to another, and forms a common sheath surrounding all the members of a hair bundle; the basal sacculle, which does not contain pigment, has stained deeply; part of the hair germ and part of the G_2 club-sheath are in the section but do not present a distinctive appearance. $\times 300$.
- Fig. 20. Follicle from dorsum of No. 72 *a* containing three zigzags, one of each of the first three generations; G_1 hair highest in the follicle, G_2 intermediate in position, G_3 lowest; similarly differences in size in the portions of the hairs shown are noticeable; below

the sheath of the G_2 hair-club is the hair germ of G_4 and below that the basal sacculle. $\times 110$.

RESIDUAL PIGMENT.

Fig. 21. Monotrich from dorsum of No. 21 *a* in very late catagen phase. Features to be noted in order downwards from the surface of the skin are: persistent region of follicle above the rather indistinct sebaceous glands; hair-club with its sheath; hair germ of G_2 above residual pigment in the basal sacculle; the remnants of the dwindling part of the root. $\times 110$.

Fig. 22. Skin from mid-dorsum of No. 123 *a*, viewed from below, showing the distribution of residual pigment masses; the latest hair generation probably G_4 ; the hair bundles are arranged in small groups. $\times 65$.

Figs. 23 to 25 are longitudinal sections of hair follicles from the mid-dorsum of No. 25 *a* to show the club-sheath, hair germ, and basal sacculle with contained pigment; stained.

Fig. 23. Large overhair; the basal sacculle, which contains only a little pigment, has stained deeply. $\times 180$.

Fig. 24. Large overhair. $\times 180$.

Fig. 25. Small overhair. $\times 180$.

Fig. 26. Hair of Type A-B from dorsum of No. 17 *a*; the base of the hair is unusual in shape and in containing pigment; part of the club-sheath is shown; not only the basal sacculle, but the hair germ also, which have been removed, contained pigment. $\times 110$.

Fig. 27. Auchene of G_2 from mid-dorsum of No. 67 *a*; just above the base of the hair is an unusual swelling containing pigment; for a little distance above this swelling the distribution of the pigment in the medulla is irregular. The basal sacculle contained pigment. $\times 110$.

Fig. 28. One of the larger vibrissae from the lip of No. 36 *a*; pigment in club; the broad aspect of the club is shown. (Compare the side view in Fig. 34.) $\times 35$.

HAIR SUCCESSION.

Fig. 29. Dissection from fore-leg of mouse of 7 weeks showing Telogen I-Anagen II boundary. The increase of length of root as the anagen area is entered is a gradual one. $\times 22$.

Fig. 30. Dissection from side of body near fore-leg of mouse of 10 weeks showing Telogen III-Anagen IV boundary. The boundary between the two areas is more sharply defined than that in the previous figure because in the shortest growing root in Fig. 30 development has proceeded further than in several of the short roots in the other figure. $\times 15$.

Fig. 31. Dissection from dorsum of 10 weeks mouse showing transition from Anagen III-Catagen III-Telogen III. $\times 22$.

SPECIAL HAIRS.

Fig. 32. Hairlet close to monotrich. $\times 45$.

Fig. 33. Bundle of three hairlets from near mamma of No. 84 *a*. $\times 45$.

Fig. 34. Dissection from lip of No. 60 *b*; basal portion of a long vibrissa, the hair-club viewed from the side; in a growing companion vibrissa the medulla is beginning to be formed; of the surrounding hairs the smallest are hairlets. $\times 22$.

Fig. 35. Very small hair from lip of No. 9 *a*; no medulla; differs from a hairlet in thickness and in curve; a companion hair is growing. $\times 45$.

Fig. 36. Dissection from convex surface of ear close to the middle of the ear margin of No. 19 *a*. $\times 45$.

Fig. 37. Group of three hairs from anterior end of dorsal surface of tail of No. 19 *a*. The middle hair has a growing companion in the early anagen phase. $\times 35$.

HAIR-TYPE ASSOCIATIONS WITHIN SINGLE FOLLICLES ON THE MAIN AREA.

The hairs are distinguished as left, middle, or right, according to the position of the hair tip in the figure. The position in which a hair is resting is stated; usually this is on the side or else flat, that is, presenting squarely the ectal or ental surface. All are from the mid-dorsum. The bundles come from various animals. All $\times 9\frac{1}{2}$.

- Fig. 38. G_1 A for comparison in length and hair curve with hairs in the later figures; side; total length .96 cm., tip .18 cm.
- Fig. 39. G_1 B (left), G_2 B (middle), G_3 B (right); all flat. It is unusual for a G_3 hair to be so much shorter than a G_2 hair in the same bundle.
- Fig. 40. G_1 D (left) side, G_2 C (right) side but with blade nearly flat, G_3 B (middle) flat.
- Fig. 41. G_1 D (middle) side, G_2 D (left) side, G_3 B (right) flat.
- Fig. 42. G_1 D (middle), G_2 D (left), G_3 D (right); all side. Each hair contains three constrictions and the basal curl.

HAIR CURVES.

Figs. 43 to 52 show the natural curve of hairs viewed from the side. All are of G_1 . The first four are from the mid-dorsum, the rest from other parts of the main area, the shortest zigzags coming from the anterior end of the head. The figures are from camera lucida drawings. Differences in thickness are not shown. All $\times 10$.

- Fig. 43. Type A; the length is .91 cm.
- Fig. 44. Type A-B.
- Fig. 45. Type B.
- Fig. 46. Type C; the blade is a long one, the constriction short. In many auchenes the bend at the constriction is more pronounced.
- Fig. 47. Type C-D; three constrictions and the basal curl.
- Fig. 48. Type D; three constrictions and the basal curl.
- Fig. 49. Type D; two constrictions and the basal curl.
- Fig. 50. Type D; two constrictions and the basal curl.
- Fig. 51. Type D; this hair is regarded as containing one constriction and the basal curl, but this figure indicates how one cannot draw a hard and fast line between a constriction and a bend at the apical end of the basal curl.
- Fig. 52. Type D; this hair is regarded as having no constriction but only the basal curl; the length is .115 cm.

THE CUTICULAR SCALES.

Figs. 53 to 122 are from camera lucida drawings of the edges of the cuticular scales. Measurements and other data for most of these hairs are given in Table I. Unless another source is stated it is to be understood that a numbered hair comes from the mid-dorsum of No. 25 a. Where two drawings are given of the same position "a" is from the ectal, "b" from the ental surface. All $\times 700$.

Type A, Figs. 53 to 67. All except Figs. 53, 54, 66 and 67 are from Hair No. 15. Figs. 55 to 65 are from the ectal surface.

- Fig. 53. Point of tip.
- Fig. 54. Middle of tip.
- Fig. 55. Apical end of shaft.
- Fig. 56. Pos. $\frac{1}{10}$.
- Fig. 57. Pos. $\frac{2}{10}$.
- Fig. 58. Pos. $\frac{3}{10}$.
- Fig. 59. Pos. $\frac{4}{10}$.
- Fig. 60. Pos. $\frac{5}{10}$.
- Fig. 61. Pos. $\frac{6}{10}$.
- Fig. 62. Pos. $\frac{7}{10}$.
- Fig. 63. Pos. $\frac{8}{10}$.
- Fig. 64. Pos. $\frac{9}{10}$.
- Fig. 65. Pos. $\frac{10}{10}$.
- Fig. 66. Apical end of base. The most proximal pigment group of the shaft is included.
- Fig. 67. Base, proximal end, showing the hair-club.

Type A-B, Hair No. 191, approaching Type A in form.

- Fig. 68 a and b. Pos. $\frac{1}{10}$, the region of greatest breadth; the ectal surface is here slightly concave.

Type A-B, Hair No. 195, about midway in form between Type A and Type B.

Fig. 69 *a* and *b*. Pos. $\frac{2}{10}$.

Fig. 70 *a* and *b*. Pos. $\frac{4}{10}$.

Fig. 71 *a* and *b*. Pos. $\frac{6}{10}$.

Type A-B, Hair No. 57, approaching Type B in form.

Fig. 72 *a* and *b*. Near Pos. $\frac{2}{10}$, the region of greatest breadth.

Fig. 73 *a* and *b*. Pos. $\frac{2}{10}$.

Fig. 74. Pos. $\frac{4}{10}$, ectal surface.

Fig. 75. Pos. $\frac{6}{10}$, ectal surface.

Fig. 76. Pos. $\frac{8}{10}$, ectal surface.

Type B, Figs. 77 to 91. All except Figs. 104 and 105 are from Hair No. 54.

Fig. 77. Point of tip.

Fig. 78. Middle of tip.

Fig. 79 *a* and *b*. Apical end of shaft; one apical vacuole in the basal end of the tip.

Fig. 80 *a* and *b*. Pos. $\frac{1}{10}$.

Fig. 81 *a* and *b*. Near Pos. $\frac{2}{10}$, region of maximum breadth.

Fig. 82 *a* and *b*. Pos. $\frac{2}{10}$.

Fig. 83 *a* and *b*. Pos. $\frac{4}{10}$.

Fig. 84 *a* and *b*. Pos. $\frac{6}{10}$.

Fig. 85 *a* and *b*. Pos. $\frac{8}{10}$.

Fig. 86 *a* and *b*. Pos. $\frac{8}{10}$.

Fig. 87 *a* and *b*. Pos. $\frac{8}{10}$.

Fig. 88 *a* and *b*. Pos. $\frac{9}{10}$.

Fig. 89 *a* and *b*. Pos. $\frac{9}{10}$.

Fig. 90. Apical end of base; the most proximal pigment group of the shaft is included.

Fig. 91. Base, proximal end, showing the hair-club.

Type B, Hair No. 194, from halfway between mid-dorsum and root of tail.

Fig. 92. A little proximal to Pos. $\frac{1}{10}$, ectal surface; the ectal furrow is deep and long.

Type B: figures to illustrate the difference in pattern in hairs from the same part of the body of different mice.

Fig. 93. Hair No. 10, the narrowest awl in fifty measured from mid-dorsum of Mouse No. 20 *a*, Pos. $\frac{4}{10}$, ectal surface.

Fig. 94. Hair No. 46, the broadest awl in fifty measured from mid-dorsum of Mouse No. 25 *b*, Pos. $\frac{4}{10}$, ectal surface.

Fig. 95. Hair No. 5, an awl of the average maximum breadth for those measured from the mid-dorsum of Mouse 25 *b*, Pos. $\frac{4}{10}$, ectal surface.

Fig. 96. Hair No. 30, a narrow awl from mid-dorsum of Mouse No. 25 *b*, Pos. $\frac{6}{10}$, ectal surface.

Type C, Hair No. 106.

Fig. 97 *a* and *b*. Pos. $\frac{4}{10}$, apical end of constriction.

Fig. 98 *a* and *b*. Middle of constriction.

Fig. 99 *a* and *b*. Basal end of constriction.

Type C, Hair No. 46, with the constriction comparatively very broad.

Fig. 100 *a* and *b*. Middle of constriction.

Type D, side. Except where stated otherwise a drawing is from Hair No. D 1. The ental surface is on the left, the ectal on the right.

Fig. 101. No. D 13. Point of tip.

Fig. 102. Middle of tip.

Fig. 103. Base of tip, apical end of shaft.

- Fig. 104. Segment I, middle.
 Fig. 105. Constriction I.
 Fig. 106. Segment II, middle.
 Fig. 107. Constriction II.
 Fig. 108. Segment III, middle.
 Fig. 109. Constriction III.
 Fig. 110. Segment IV, broadest part.
 Fig. 111. Basal curl; narrowest part of the one pronounced bend.
 Fig. 112. Basal curl; just proximal to last figure.
 Fig. 113. Base of shaft, apical end of base.
 Fig. 114. Hair not numbered. Base, showing hair-club.

SPECIAL HAIRS.

- Fig. 115. From lower lip, Hair No. 001; middle of hair, ental surface; the scale edges of the ectal surface are drawn as dotted lines.
 Fig. 116. From ear, Hair No. 02; near basal end of shaft, broadest part of hair, ental surface.
 Fig. 117. From tail, Hair No. 197, Pos. $\frac{1}{16}$, the broadest part of the hair, ental surface.
 Fig. 118. Vibrissa, Hair No. W 5, from snout; not orientated; middle of shaft. Distal to the part of the hair shown in this figure the margins are wavy but further apart.
 Fig. 119. Vibrissa, Hair No. W 5; about .03 cm. from basal end of shaft, the region of greatest breadth.
 Fig. 120. Vibrissa, Hair No. W 5; about .02 cm. from basal end of shaft.
 Fig. 121. Hairlet; apical part; a spiral scale margin is shown.
 Fig. 122. Hairlet. Hair No. M 1, from mid-dorsum of No. 20 a; middle of hair.

DESCRIPTION OF CHARTS.

The order of appearance of the hairs of generations I, II, and III on the main area.

It will be obvious at a glance how the skins have been cut, along the mid-ventral line and on the limbs. The skins are drawn in three arbitrary sizes according to the hair generations present: (1) G_1 only, (2) G_2 but not G_3 , (3) G_3 . Any special hairs, including those near mammae in females, situated within the main area are omitted. On the legs the charts stop a little short of the boundary between the main area and the areoles; these parts of the limbs are dealt with in the text. The tail and sub-caudal areas are omitted. The areoles of the snout, eyes, and ears are left blank. The sex of animals is not stated, no differences between the sexes in the features depicted in the charts having been detected.

Anagen areas, which have hair-roots containing pigment, and so appear dark, are shaded.

Catagen areas have vertical dashes; the hair-roots are more or less long but all the pigment, apart from any residual pigment, has passed into the hair, and consequently in such an area one notices the appearance of parallel lines produced by the margins of the roots.

Telogen areas, which contain club-hairs arranged in rows, have horizontal rows of dots.

- Chart 1. Mouse No. 11 b. G_1 is growing throughout; Catagen I reached on small areas.
 Chart 2. Mouse No. 16 a. G_1 has completed its growth on some areas, elsewhere is in late growing stages.
 Chart 3. Mouse No. 19 a. The growth of G_1 is somewhat further advanced.
 Chart 4. Mouse No. 20 a. G_1 still in the catagen phase on a small part of the body, G_2 in early growing stage near the fore-legs.
 Chart 5. Mouse No. 27 b. Growth of G_1 nearly completed. G_2 in early growing stage on large area on venter and sides. Anagen II area and Catagen I area contiguous to some extent on the posterior part of the body, and one small catagen area on the venter has been surrounded by growing G_2 hairs.

Chart 6. Mouse No. 36 *a*. Growth of G_2 well advanced. All areas are G_2 except those on the hind-legs and root of tail marked G_1 .

Chart 7. Mouse No. 48 *b*. G_2 all but finished growing. G_3 just beginning to grow in tiny patches.

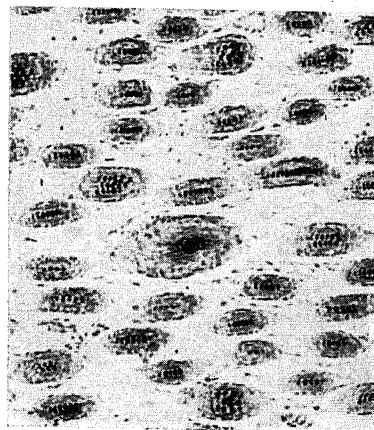
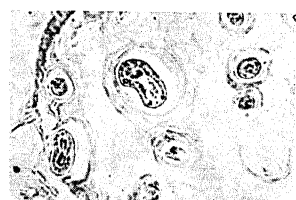
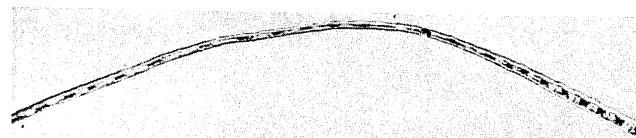
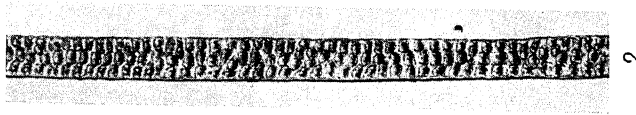
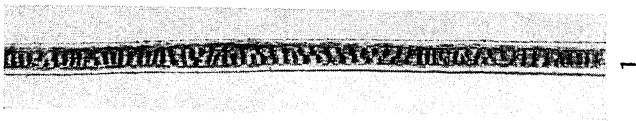
Chart 8. Mouse No. 54 *c*. G_3 growing on a considerable area of the body. Telogen III reached on a tiny area on the ventral surface of the head.

Chart 9. Mouse No. 78 *a*. The growth of G_3 well advanced. G_4 growing near the fore-legs and in patches on the abdomen. On the dorsal surface of the head and on part of each hind-leg G_3 has not yet begun to grow.

Chart 10. Mouse No. 66 *a*. The growth of both G_3 and G_4 is further advanced than in the previous chart, but Telogen II areas persist on the head and at the root of the tail. There is a Telogen II-Telogen III boundary on each side of the head.

Chart 11. Mouse No. 84 *a*. The growth of G_3 is far advanced, but a small Telogen II area still remains on the head. G_4 has made a little progress.

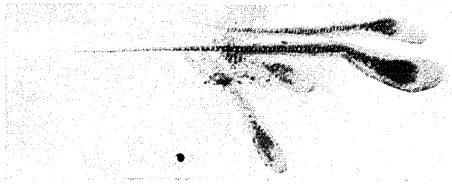
Chart 12. Mouse No. 60 *a*, a poorly grown animal showing unusual features that are dealt with in the text.



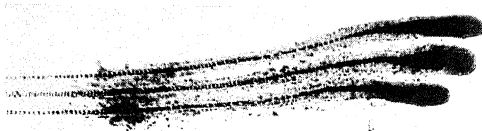
Figs. 1—12.



13



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16



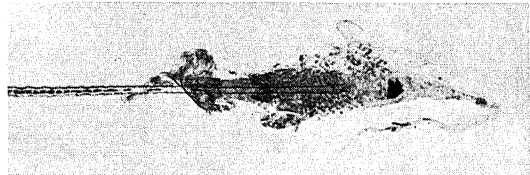
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19



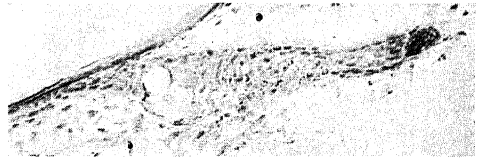
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22

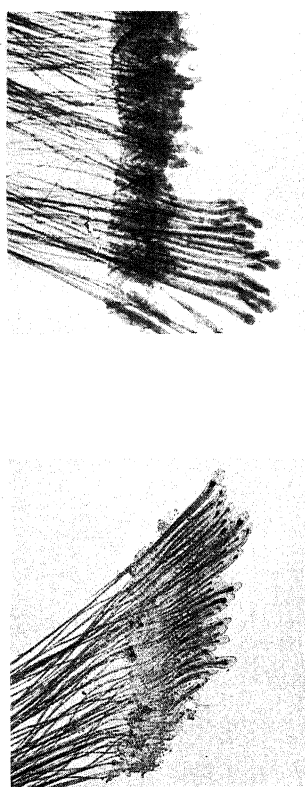


23



24



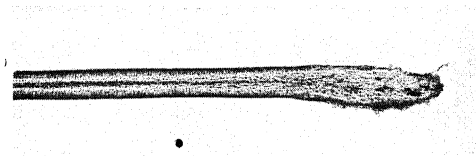
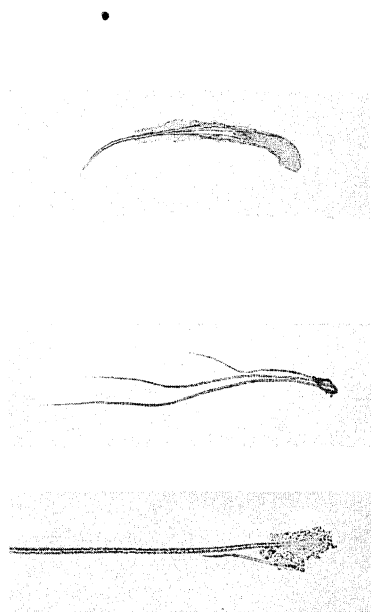


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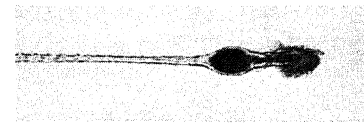
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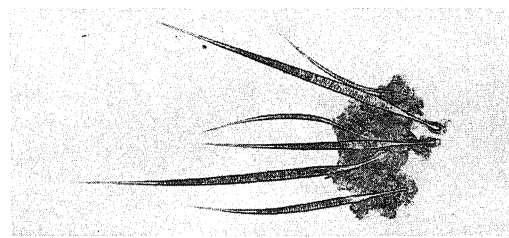
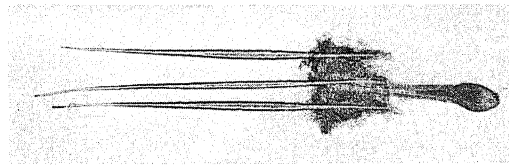
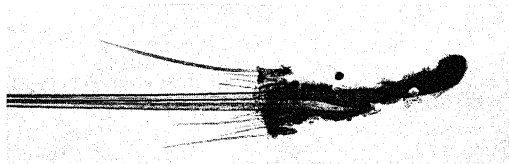
28



27



26



Figs. 26—38.

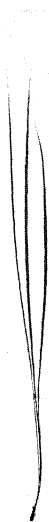


Fig. 39.

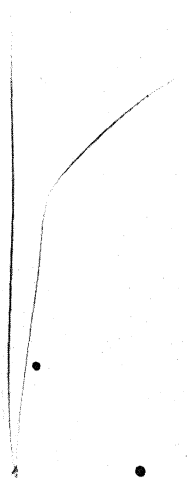


Fig. 40.

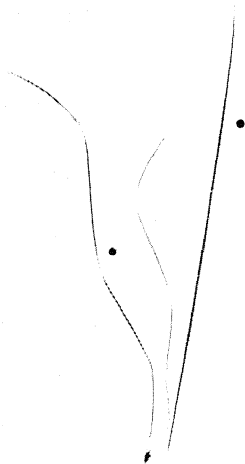


Fig. 41.

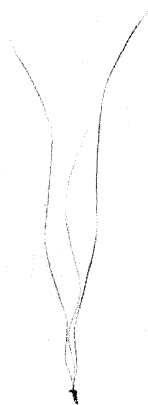
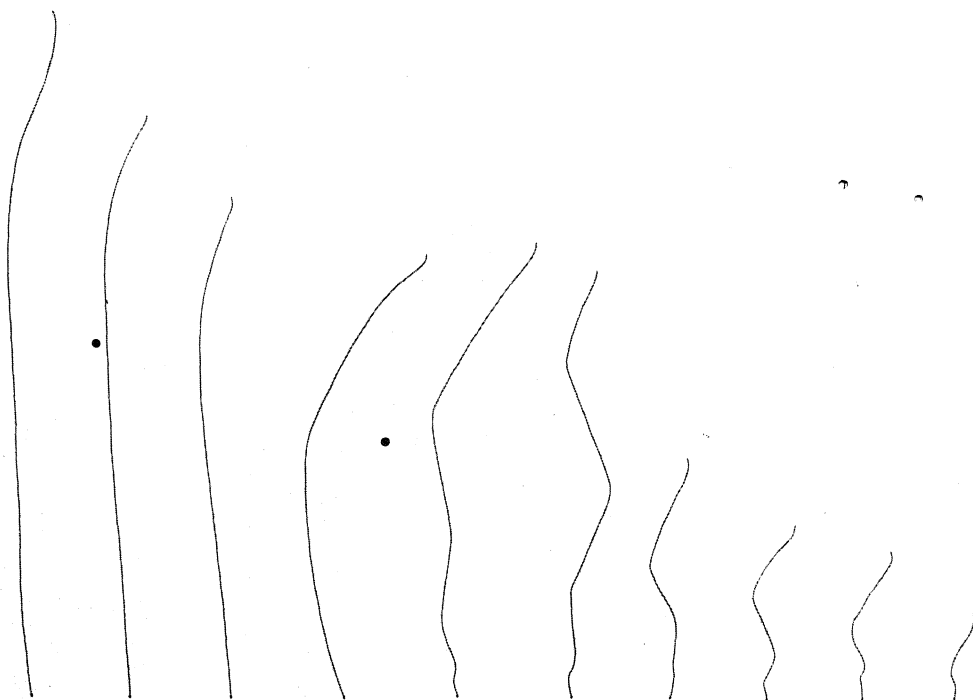
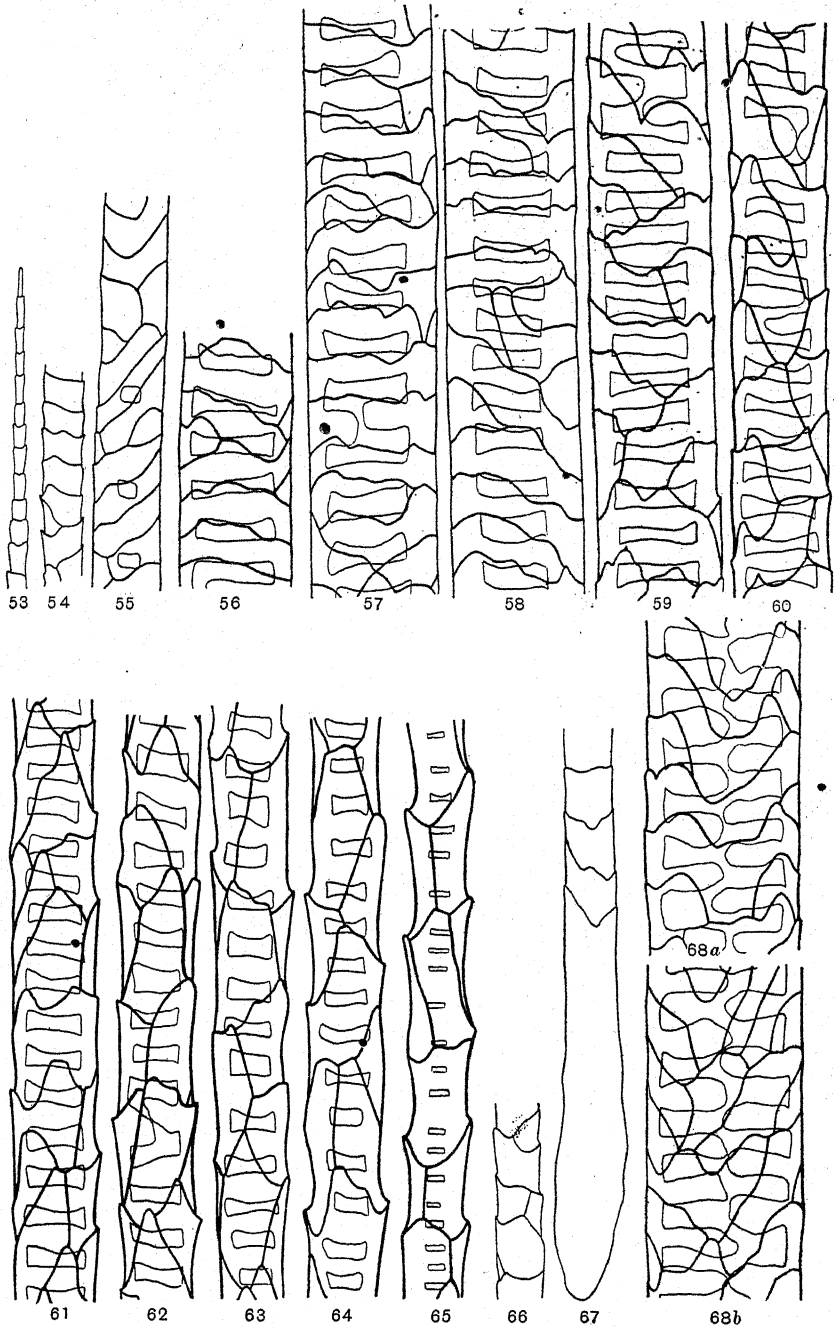


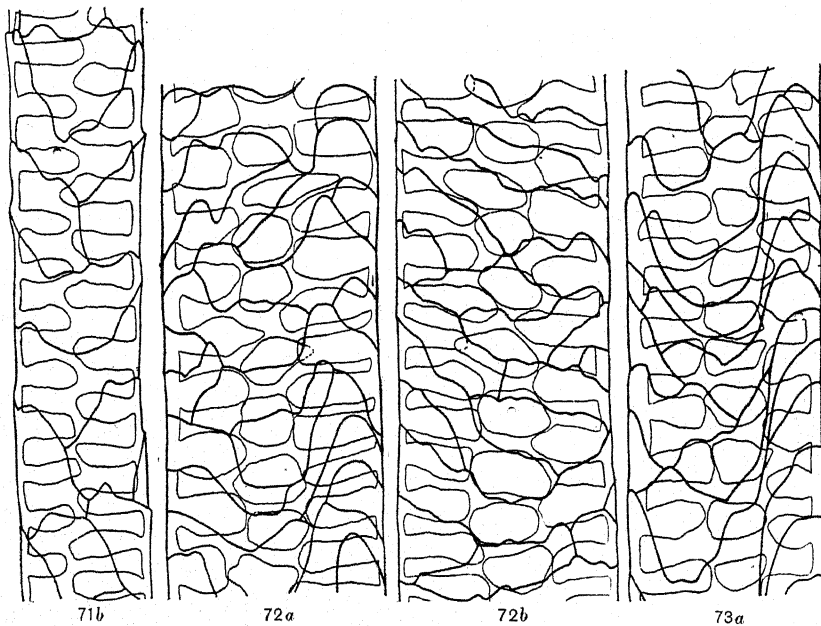
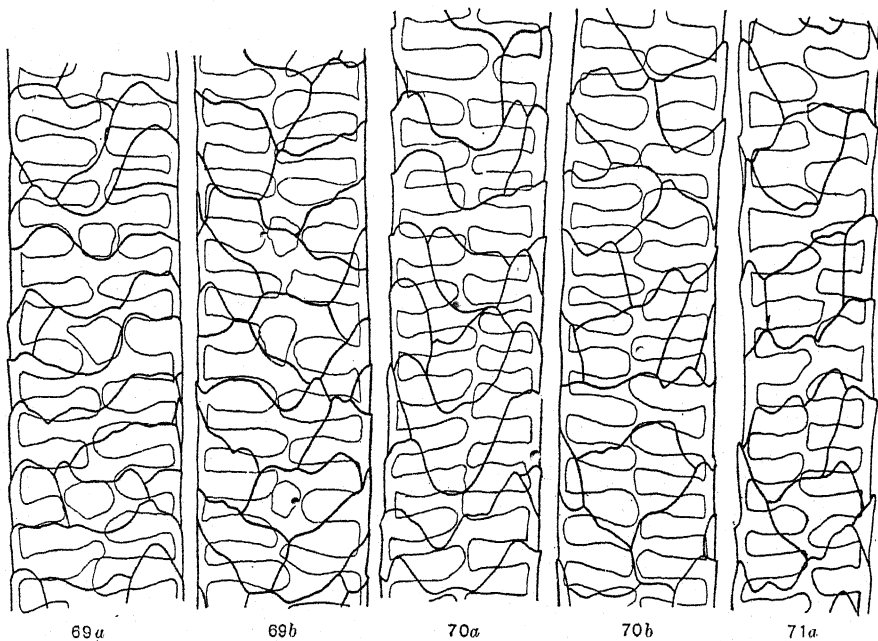
Fig. 42.



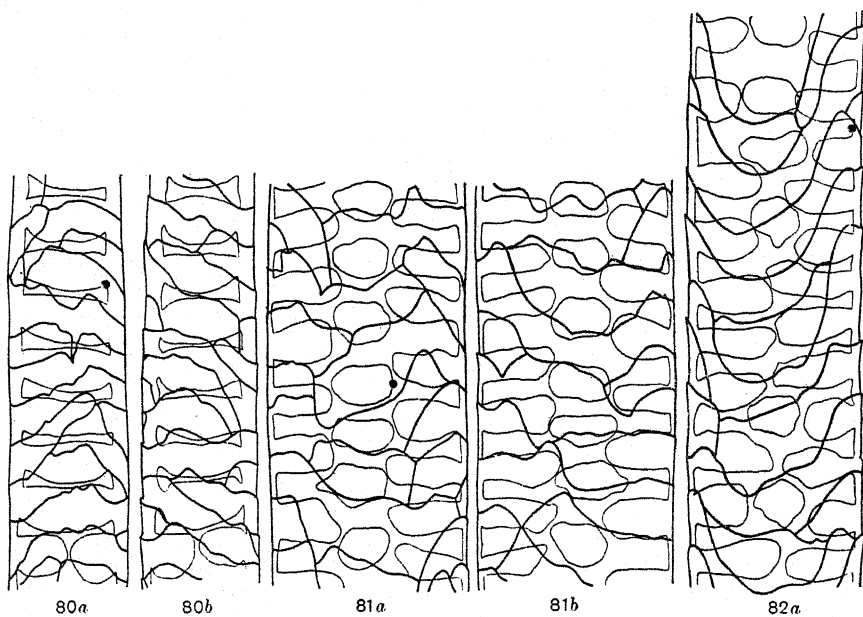
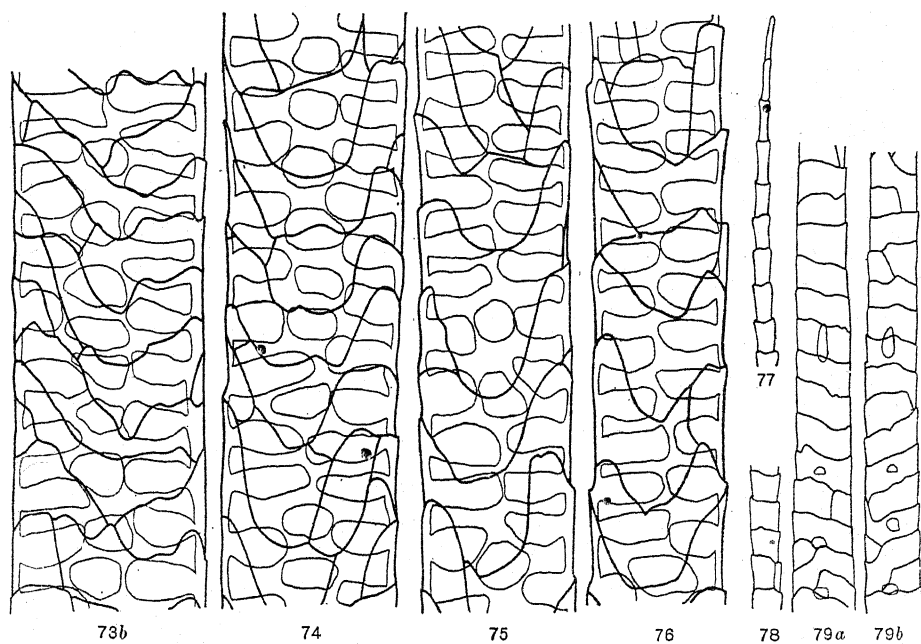
Figs. 43—52 (left to right). Longest hair Fig. 43, shortest hair Fig. 52.



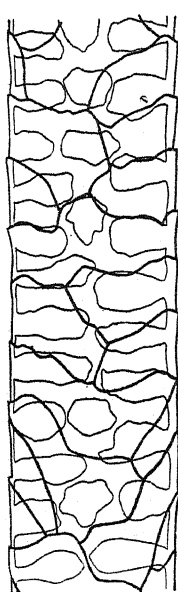
Figs. 53—68 b



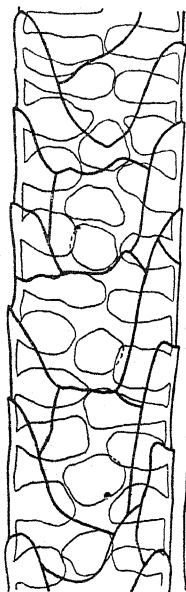
Figs. 69 a—73 a.



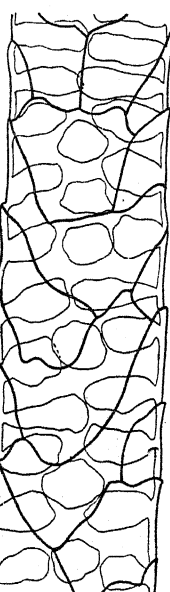
Figs. 73 b—82 a.



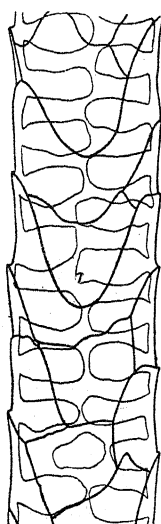
82b



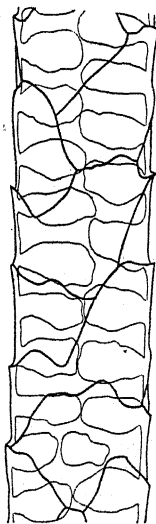
83a



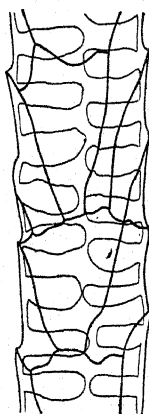
83b



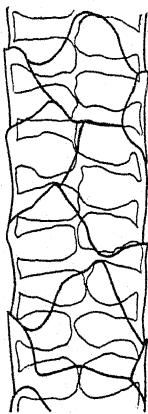
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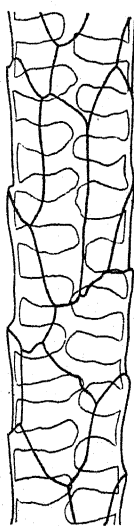
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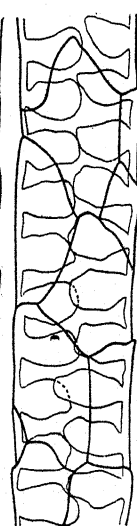
85a



85b



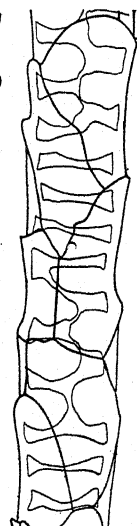
86a



86b

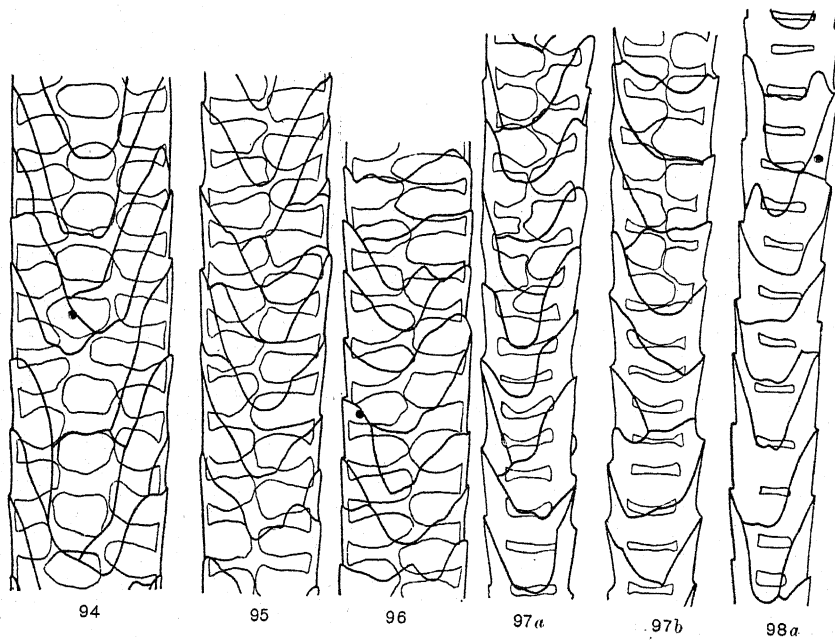
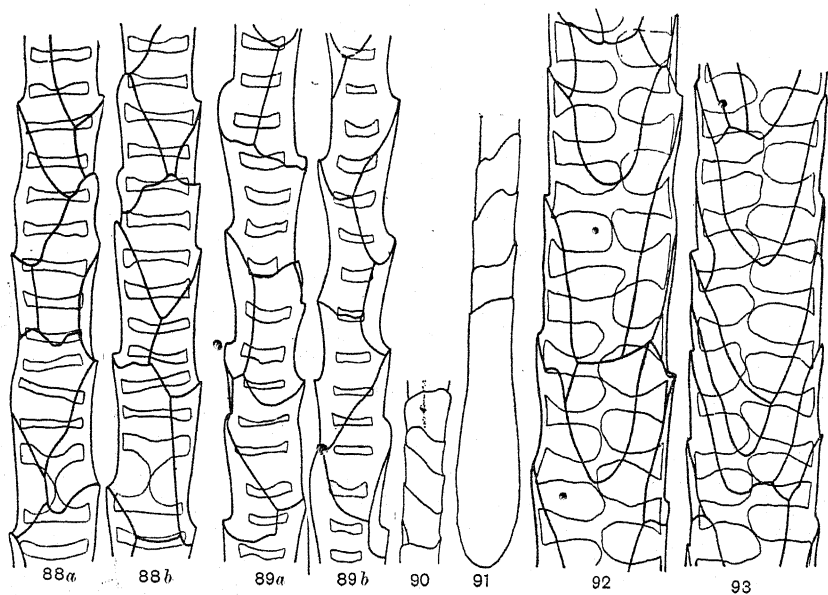


87a

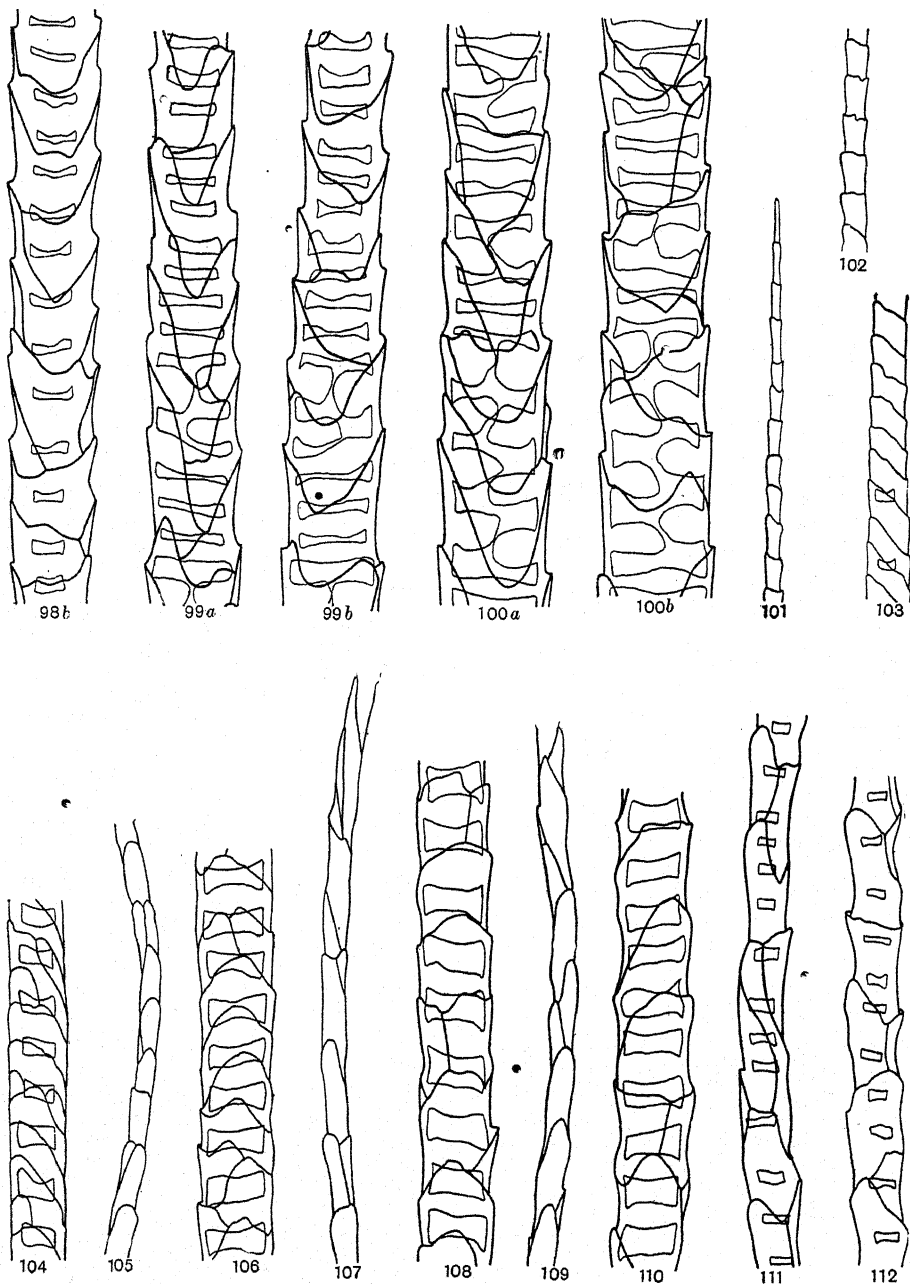


87b

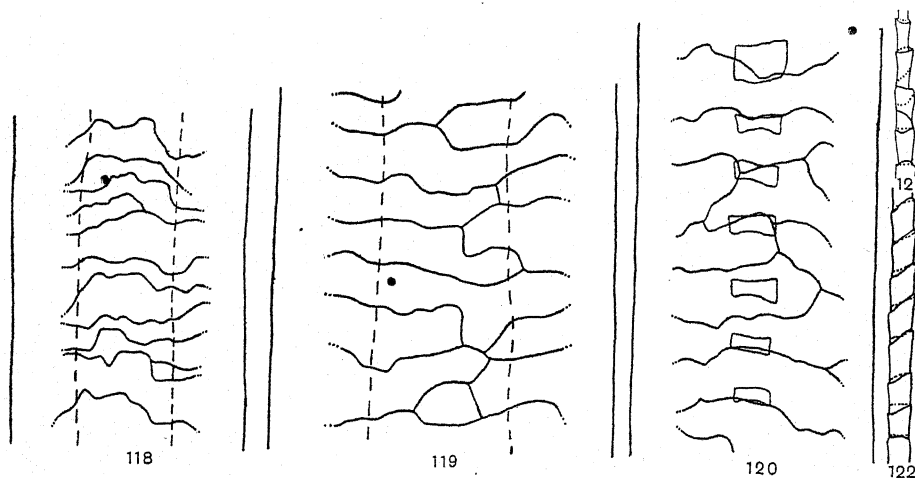
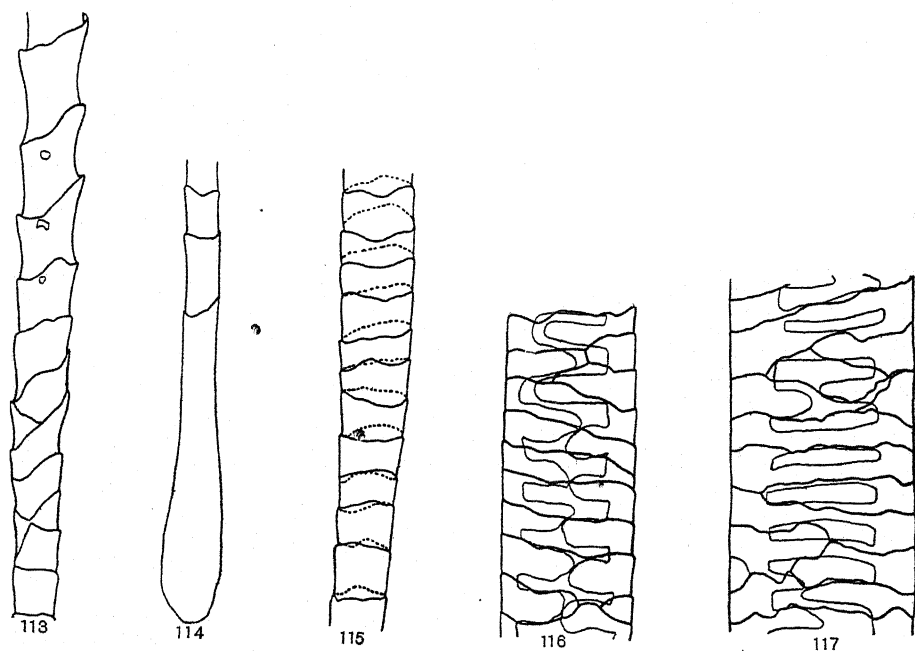
Figs. 82 b—87 b.



Figs. 88 a—98 a.



Figs. 98 b—112.



Figs. 113—122.

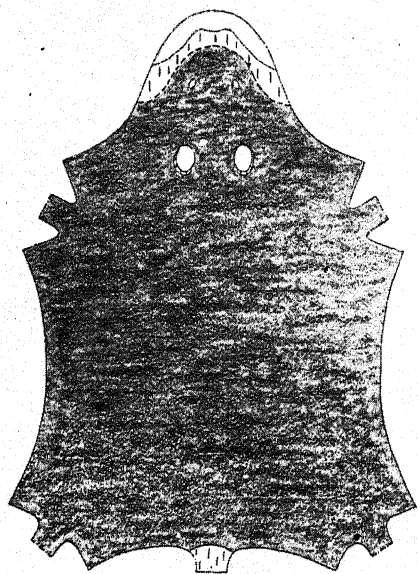


Chart 1

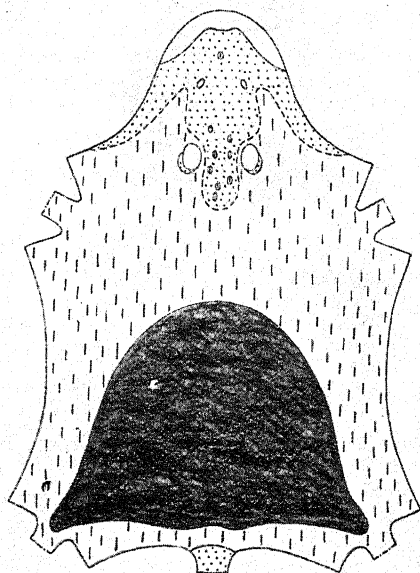


Chart 2.

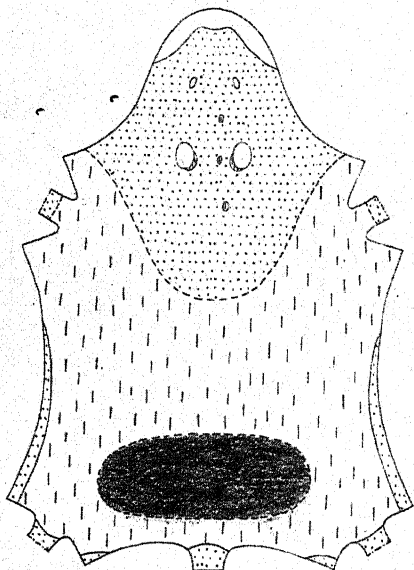


Chart 3.

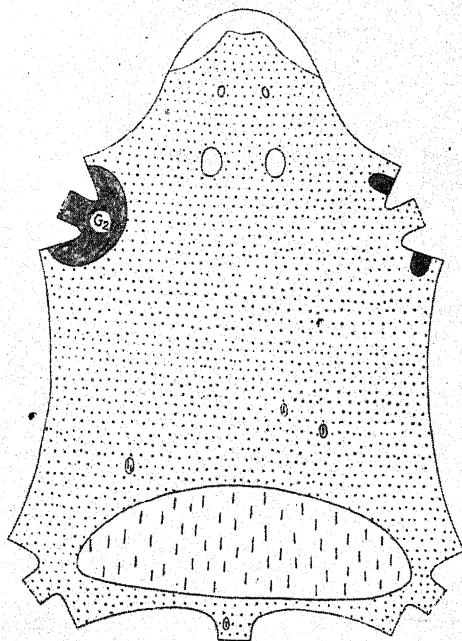


Chart 4.

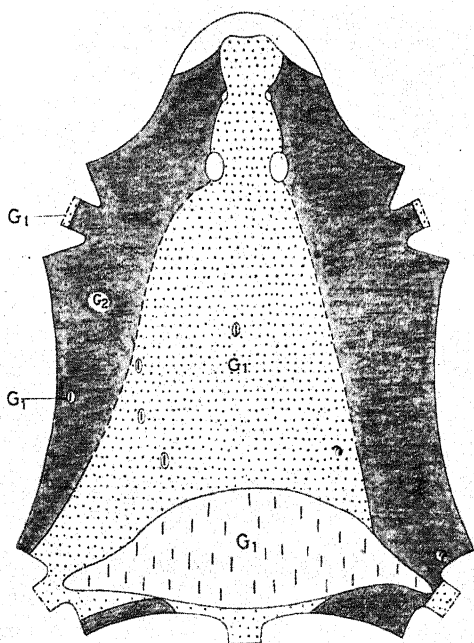


Chart 5.

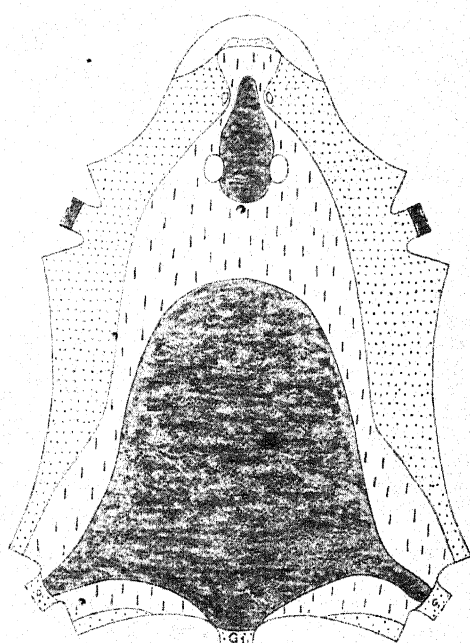


Chart 6.

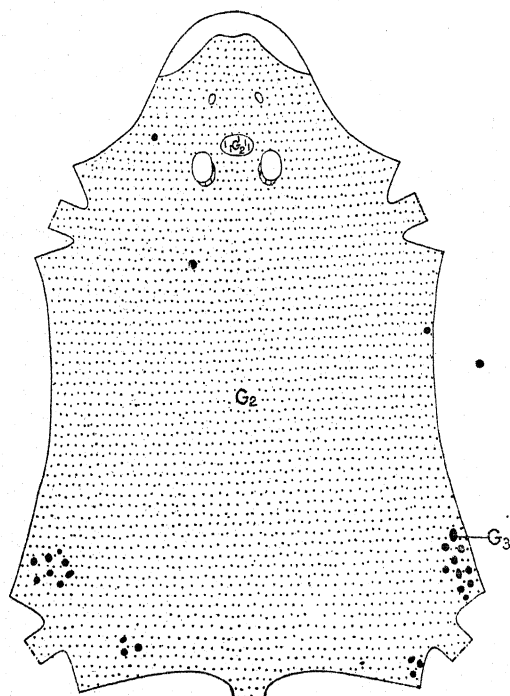


Chart 7.

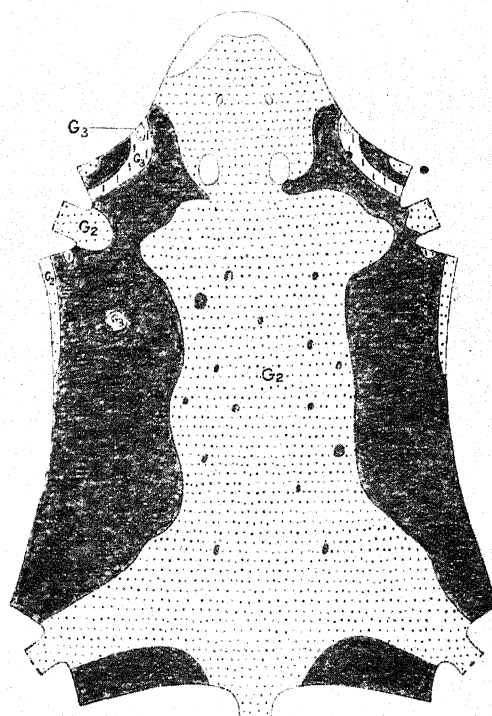


Chart 8.

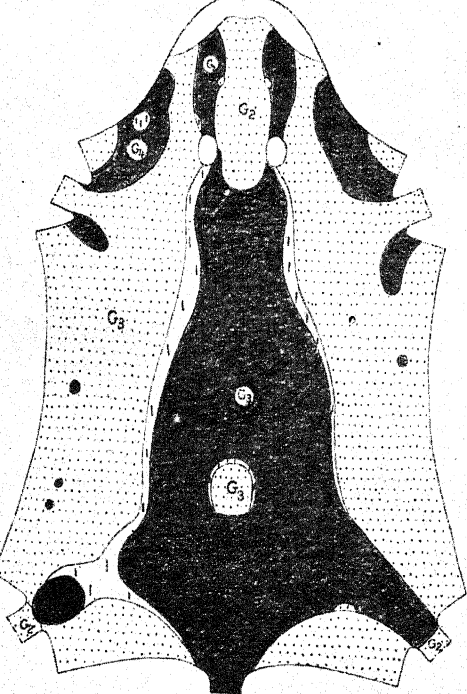


Chart 9.

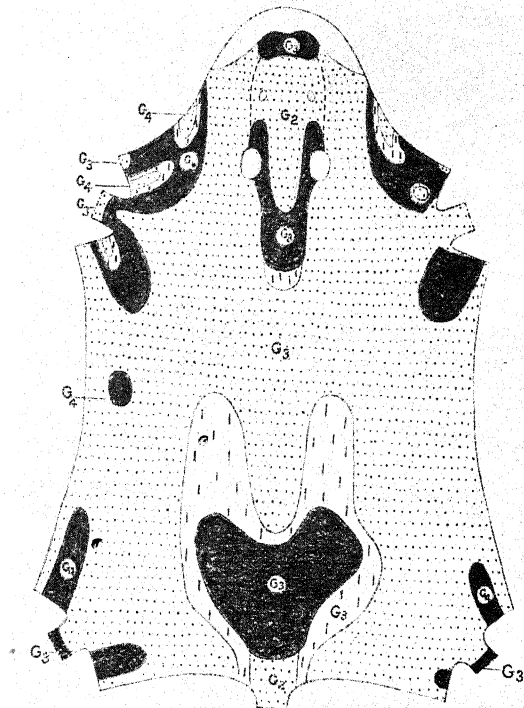


Chart 10.

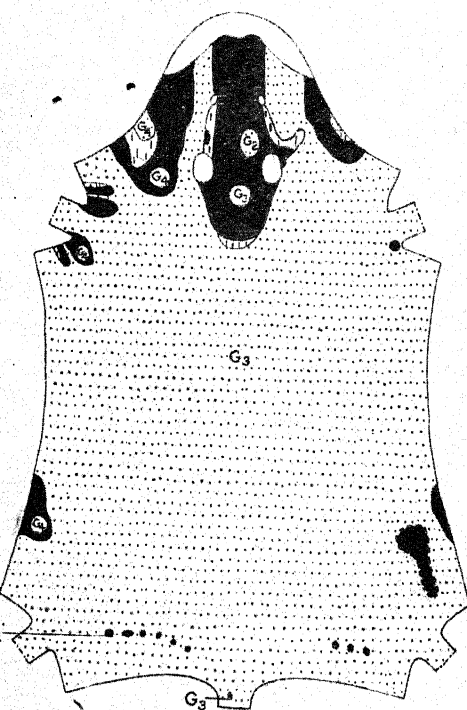


Chart 11.

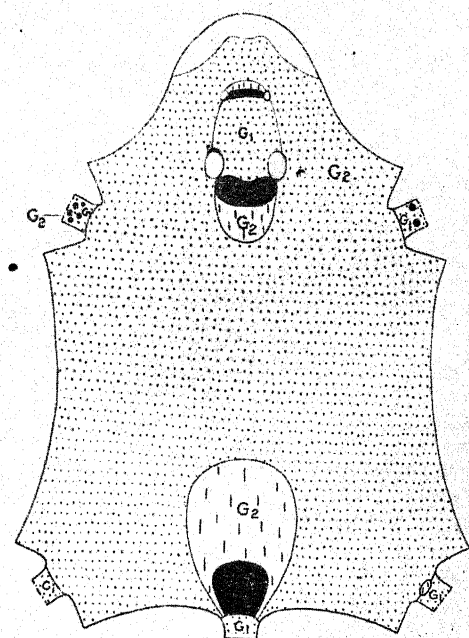


Chart 12.

CAMPANULA PERSICIFOLIA AND ITS TETRAPLOID FORM, "TELHAM BEAUTY."

By A. E. GAIRDNER.

(The John Innes Horticultural Institution.)

(With Four Text-figures, Six Tables and Three Plates.)

THE large form of *Campanula persicifolia* known as Telham Beauty was first exhibited by Messrs Barr at Holland House in July, 1916¹. The original plant was raised by Mr F. D. Thurston, gardener at Telham Court, and was said to be a seedling from *C. persicifolia*, "possibly a cross with *C. turbinata*," the latter supposition is however improbable. It received an Award of Merit from the R.H.S. Floral Committee and is described as having "large, open, shallow cup-shaped flowers about 2½ in. across and pale lilac-mauve in colour²."

Bot. Mag. 1795, Pl. 397, figures a plant described as *C. persicifolia* var. *maxima*, which is evidently the same as Telham Beauty. It is there said to have come "from South Carolina" in 1791, but doubtless from a horticultural origin. The plant has been long ago lost and its spontaneous reappearance is of interest.

Some plants of Telham Beauty were obtained by this Institution in 1918 and efforts were made to cross it with the ordinary *C. persicifolia*, with the dwarf form "*nitida*" and with *C. grandis*. The only plant obtained from Telham Beauty × "*nitida*" was a plant with much doubled flowers, which set no seed and died in 1922.

The variety "*nitida*" is a most peculiar plant standing about 4-5 in. high, characterised by small, thick, very dark green leaves, which has arisen here as a recessive in various crosses made between plants of *C. persicifolia*. It occurs in nurserymen's lists under the name of *Campanula nitida*, and the fact that it has been popularly regarded as a distinct species is not surprising, for in general appearance it is utterly different from the type. Not the least of its peculiarities consists in the relations of the ovary to the perianth. By some curious change in the relative development of the parts the ovary, instead of being simply inferior, projects upwards in the centre of the flower, so much so that with scarcely any exaggeration it might be described as superior.

¹ *Gardener's Chr.* 1916, LX. p. 88.

² *Journ. R.H.S.* 1917, XLII. Proc. p. clxv.

Aiton. Hort. Kew.¹ gives a description of *C. nitida* which is not full enough for identification but refers to Dodart, 1676², where there is a plate showing a plant probably identical with ours under the name *Trachelium Americanum minus*. In the description the passage "le calice et formé comme une étoile au milieu de laquelle le pericarpe s'élève divisé en trois" evidently refers to the semi-superior position of the ovary which is characteristic of our plant. The Kew Index appears to be in error in referring *C. nitida* to *C. Parryi* Gray.

In its essential features "*nitida*" breeds true, though like the parent type it is self-sterile. We have had it both single and double, in several shades of blue and white, and with corollas of various shapes.

As it was thought possible that Telham Beauty might be a tetraploid form, Professor Matsui, who was working at this Institution in the spring of 1920, was asked to look into the cytology of the two forms. He reported Telham Beauty to have 32 somatic chromosomes and *C. persicifolia* about 18. The latter number was later found by Marchal (1920) to be 16³. In view of the record in *Bot. Mag.* we may feel every confidence that this is at least the second occasion on which the tetraploid has arisen.

At this point the experiments were taken over by the present writer.

In appearance Telham Beauty is a much more robust plant than the type, the flower stem reaching 3 ft. in height as against 2 ft., and both the rosette and stem leaves are larger and thicker in texture. The most striking difference, however, is in the flower, *C. persicifolia* having a deep bell-shaped corolla, while that of Telham Beauty is much larger and shallower. *C. persicifolia* in its ordinary forms has always been found by us to be strictly self-sterile, but greatly to our surprise Telham Beauty proved to be self-fertile. Offspring so raised from it have been a heterogeneous lot, with corolla-shapes ranging from the large saucer form of Telham Beauty to small almost sessile flowers opening flat and with stigmas spreading somewhat like those of *C. grandis*.

No special attention has been paid to the *persicifolia* plants used in these crosses. At first a white single, a white double, and a bluish white slightly double were used, and subsequently back crosses were made with a white single. Some of the less deep shades of blue proved to be heterozygous for white, but these observations relate only secondarily

¹ Aiton. Hort. Kew. Vol. I. p. 346.

² Dodart, *Mémoire pour servir à l'Histoire des Plantes*, 1676, p. 119.

³ Marchal, C., *Recherches sur les Variations Numériques des Chromosomes*, Bruxelles 1920.

to somatic characters and the factorial constitutions of the several shades have not been determined.

As regards flower colour Telham Beauty is evidently not homozygous for blue. The plants produced by self-fertilisation were 29 blues of various shades and 2 whites. For a tetraploid plant of constitution BBbb the simplest expectation, on the hypothesis of free mating between the factorial elements is, as Blakeslee¹ has argued, a recessive in 36, with which this result agrees fairly well.

Twenty-one F_1 plants have been raised from Telham Beauty $\varphi \times C. persicifolia$ σ , but so far seed from the reciprocal cross has failed to germinate, although it looks quite good. Crossed with "*nitida*" we have had a few plants made each way. Only 5 out of 14 F_1 plants tested set seed with their own pollen, but by intercrossing them we have raised a considerable F_2 .

Both parents have been used in back crosses with the F_1 and the following families were raised and planted out, though not all survived to flower.

Cross	No. of families	No. of plants
$C. persicifolia \times F_1$	2	26
$F_1 \times C. persicifolia$	5	66
$F_2 \times C. persicifolia$	3	102
Telham Beauty $\times F_1$	6	34
$F_1 \times$ Telham Beauty	8	49
Telham Beauty $\times F_2$	3	5
$F_2 \times$ Telham Beauty	3	23

The shape and size of the F_1 flowers were very various; 11 approached Telham Beauty in shape, but only 3 resembled it in size, 2 had the bell shape of *C. persicifolia*, while 5 had an intermediate or rather wedge shape.

The pollen of various plants has been examined and percentages of bad grains roughly estimated. That of *C. persicifolia* is fairly even in size, with about 32 per cent. bad grains, while that of Telham Beauty has a slightly higher proportion of bad grains and they are much more variable in size, some being considerably larger than those of the type. In the F_1 bad pollen varied from 25 per cent. to over 60 per cent., most plants showing at least a few of the large grains.

The number of pores in the walls of the fully developed pollen grains is variable, the diploids having two or three and the tetraploids up to five.

¹ Amer. Nat. 1921, LV. p. 260.

Cytology.

As already stated, *C. persicifolia* has 8 gametic and 16 somatic chromosomes (see Text-fig. 1), while Telham Beauty has the tetraploid numbers 16 and 32. The size of the chromosomes in the two forms is approximately the same, but the nuclei in the megaspore mother-cells and pollen mother-cells of Telham Beauty are rather more than twice as large as those of *C. persicifolia* type. Root-tips, fixed in strong Flemming *plus* an equal quantity of distilled water and stained with iron-alum haematoxylin, were used in every case for the somatic counts. A noticeable point in the root-tip material is the large number of nucleoli in the resting nuclei of the forms with the largest number of chromosomes. A rough count gave for the diploid 90 per cent. nuclei with 1 nucleolus and 10 per cent. with 2, and for the tetraploid 55 per cent. with 1, the remainder having from 2 to 5 nucleoli.



Fig. 1. *C. persicifolia*, metaphase plate from root-tip.

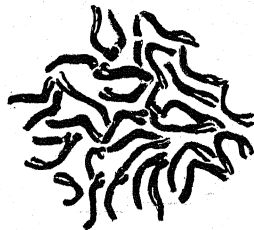


Fig. 2. Telham Beauty, metaphase plate from root-tip.

• Pairing of the somatic chromosomes is generally clear in the diploid form, and may also be made out in the tetraploid, though it is not so easy to follow here. As can be seen from Table II, 9 of the F_1 plants from Telham Beauty \times *C. persicifolia* had 24–5 chromosomes for the somatic number, while one had (?) 32. Of the Telham Beauty \times “*nitida*” hybrids 7 had 24 and 2 others had (?) 28–30 and (?) 16 respectively, while from the reciprocal cross only two plants have been examined, 1 had 24 and the other 32, the latter being plant $\frac{14^3}{21}$ which is described in detail later. In the F_2 the chromosome numbers have ranged from the 16 of the diploid parent up to at least 30, about half the plants examined having numbers lower than the F_1 and half higher.

In the type, reduction takes place quite normally, 8 pairs of chromosomes lying side by side in diakinesis, or forming rings. After the first division a membrane is formed round the daughter nuclei before they proceed to the second division. Regular tetrads are formed developing

into pollen grains with 2 or 3 germ pores. In the tetraploids the reduction division is much less regular. At the first division laggards may be seen on the spindle, and in the polar view of the first anaphase one or two pairs of chromosomes are often seen quite apart from the main plate. These may form the nucleus of a small cell or they may divide again forming 2 still smaller cells and in this way instead of the normal tetrad as many as 7 or 8 cells develop. The relative proportion in which these abnormalities occur may be seen by the following numbers of cells seen inside the pollen mother-cell wall in material taken from two tetraploid plants.

Three cells 4 times, 4 cells 21 times, 5 cells 10 times, 6 cells 10 times. Sometimes one or both of the daughter nuclei fail to complete the second division, and in these ways pollen grains with very variable numbers of chromosomes are formed. In a Telham Beauty plant received from Kew tetrads were as a rule normal, but Plate XXVI, fig. 14,



Fig. 3. "*Nitida*," metaphase plate from root-tip.



Fig. 4. Telham Beauty \times "*nitida*," metaphase plate from root-tip.

shows an abnormality. It is drawn from a pollen smear and shows the contents of one cell drawn separately for clearness. One-half of the chromosomes are completing their second division, while the other 16 split chromosomes will probably round up into a pollen nucleus without further division. The same condition is frequently met with in later generations from the cross.

In diakinesis of the F_1 mother-cells univalents, bivalents and trivalents have been seen. It has not been possible so far to determine with how much regularity the different groupings occur. In the metaphase plate the sizes of the chromosomes only differ slightly, but in the longitudinal view of the first division spindle the different combinations are again distinguishable. The number of chromosomes which travel to either pole appears to be a matter of chance; as many as 15 have been seen in one anaphase plate, with 9 in the other. Sometimes one chromosome reaches the pole considerably earlier than the rest; probably this is a univalent which has not divided.

Division of the megaspore mother-cell is much the same as in the pollen mother-cell, that is to say in the F_1 there are univalents and bivalents and irregular distribution of the univalent chromosomes to the poles (Fig. 11) with no sign of such polarity as was described by Täckholm in *Rosa*¹.

Plant $\frac{8}{22}$. This F_2 plant with 17 somatic chromosomes has been examined in detail. At diakinesis in the pollen mother-cell division there are 8 pairs and 1 odd chromosome. Generally the first anaphase plates contain 8 and 9 chromosomes respectively, but 9 + 9 and 8 + 10 have also been counted at this stage. Presumably here the odd chromosome has divided, in the latter case both halves going to the same pole. This plant set no selfed seed, but with pollen from *C. persicifolia* it was fully fertile and 4 of the resulting plants were found to have the normal diploid number of chromosomes which paired closely in diakinesis. Crossed both ways with another F_2 which had (?) 20 chromosomes, families of 15 and 20 plants were raised, 4 of which had again only 16 chromosomes, while a fifth had 8 or 10 haploid. Pollinated by Telham Beauty only one seed germinated and the root-tips have 30–32 chromosomes, an unexpectedly large number.

Plant $\frac{14^3}{21}$. As this plant is unique in several ways it will be dealt with separately. A dwarf white diploid was pollinated from Telham Beauty and 3 plants were raised, the family $\frac{14}{21}$. 14^1 was a bluish white, 14^2 a dark blue, and one of these had 24 chromosomes, while $\frac{14^3}{21}$ was a heterozygous blue with flowers the size and shape of Telham Beauty and having 32 chromosomes. This plant on selfing gave 18 normally tall plants and 2 dwarfs. As regards flower colour the breeding results were consistent with the supposition that the factorial composition of the plant was *Aaaa*—i.e. on selfing it gave 5 blue, 9 heterozygous blue, 5 white, the 1 : 2 : 1 ratio which Blakeslee² expects from random mating of the chromosomes in a tetraploid of this composition. On back crossing with a Telham Beauty, which probably was duplex for the dominant factor (*AAaa*), it gave 24 blue and 3 white, not far off the expected ratio 11 : 1 (i.e. 33 : 3). This result leads us to suppose that 14^3 must have received *aa* from its mother's side by some doubling of the chromosomes in the egg-cell.

¹ Täckholm, 1922, *Zyt. Stud. u. d. Gat. Rosa*, p. 220.

² Blakeslee, 1921, *Am. Nat.* Vol. LV.

From the reciprocal cross to that which produced $\frac{14}{21}$ 3 plants were raised, 2 of which had 24 chromosomes, while the third had 16, the only case in which a diploid arose in the F_1 .

The pollen of $\frac{14^3}{21}$ is almost all good, only 10 per cent. bad grains having been counted, but it is uneven in size. Unfortunately no good material was obtained showing the early stages of meiosis, but reduction must take place fairly regularly as 3 of the plants raised by self-fertilisation had 30–32 chromosomes. Two of these plants, a tall and a dwarf, are the most self-fertile plants we have had¹.

Other crosses between diploids and tetraploids.

Though, as stated above, the cross between Telham Beauty and “*nitida*” was fertile whichever was taken as mother, that between *persicifolia* and Telham Beauty succeeded only when Telham Beauty was the mother. The latter result recalls the experience of Blakeslee and Belling with *Datura*, who also found the diploid fail when fertilised by the tetraploid. With *Oenothera Lamarckiana* “*Gigas*” de Vries was successful in crossing the diploid with tetraploid both ways, as I was with Telham Beauty and “*nitida*.” With *Primula sinensis* in many hundreds of attempts made here to cross the tetraploids and diploids together only 3 plants have been raised. These all came from the tetraploid as mother, but no significance should yet be attached to this coincidence.

Telham Beauty $\times \frac{14^3}{21}$ gave 28 plants, only 1 of which has been examined cytologically and has (?) 32 chromosomes.

In one case, $\frac{14^3}{21} \times$ “*nitida*” has given a normal F_1 , the 5 plants all having 23–4 chromosomes. At diakinesis in 1 of these plants the arrangement of the chromosomes was seen to consist of 6 rings, at least 3 single chromosomes and 2 strings of 3; it cannot be said that this arrangement is constant, but the strings of 3 chromosomes have been seen on various occasions and also the paired rings and single chromosomes. The number of chromosomes that go to either pole at the first division is a matter of chance as 10, 11, 12, 13 have been counted in the first or second anaphase.

When another “*nitida*” plant, $\frac{26}{21}$, was used to pollinate $\frac{14^3}{21}$ the

¹ And 5 of their descendants have also (?) 32 chromosomes.

result was different, 2 of the plants thus raised having 28-9 somatic chromosomes, while 3 others have 15-16 haploid.

On the whole the plants with the largest number of chromosomes are nearest to Telham Beauty in vegetative growth, the rosette leaves are large and thick and the whole plant stouter than the diploid forms. It must, however, be noted that "*nitida*" types have now been made with 30-2 chromosomes. Only one of these has flowered so far; the leaves are rather larger than the diploid "*nitida*," but the flower stems were only about 7 in. high. Semi-dwarfs with thick stocky stems about 18 in. high have also come in our cultures. One of these was examined cytologically and found to have (?) 27 chromosomes in the somatic cells.

In the cases where Telham Beauty \times *C. persicifolia* ($\frac{5^1}{21}$) and Telham Beauty \times "*nitida*" ($\frac{6^1}{25}$) have each given 2 plants with 32 and 24 chromosomes respectively, the most probable explanation is that the irregularities have occurred in the reduction division of the megaspore mother-cell resulting in the formation of a gamete with 24 chromosomes instead of 16, and that this has been fertilised by a normal pollen grain with 8 chromosomes from the diploid. In the opposite case where "*nitida*" \times Telham Beauty also gave sister-plants with 24 and 32 chromosomes, it has been shown by breeding that the latter plant $\frac{14^3}{21}$ must have got the double set of chromosomes from the mother which here was the diploid. $\frac{14^3}{21}$ crossed with the same "*nitida*" plant ($\frac{26}{21}$) as was used in the case of $\frac{6^1}{25}$ gave a large family, of which five had at least 28 somatic chromosomes. Root-tip counts from $\frac{26}{21}$ have given only 16 chromosomes, but further examination will be needed to discover if any of the flowering stems are tetraploid sports as is alleged to have been the case in *Primula Kewensis*.

Blakeslee and Belling¹ have also had chimaeras as regards chromosome numbers among their *Daturas* (1924). The same workers found that tetraploid *Daturas* pollinated by diploids produced many diploid plants. We have only had one such case, viz. in the reciprocal cross from that which produced $\frac{14^3}{21}$.

¹ Blakeslee and Belling, 1924, *Science*, N.S. LX. p. 19; 1924, *Am. Nat.* LVIII; 1923, *Bot. Gaz.* LXXVI. p. 329.

In the tetraploid no evidence of secondary association of the bivalents forming quadrivalents has been observed. Belling⁽¹⁾ distinguished between tetraploidy and polyploidy on the ground that true tetraploids with 4 identical chromosomes formed quadrivalents in diakinesis at metaphase, while in polyploid forms only bivalents formed. However, in *P. sinensis*, which is tetraploid in the sense of having 4 strictly homologous chromosomes, the formation of quadrivalents is the exception. It is not therefore possible to draw any conclusion from the absence of quadrivalents as to whether Telham Beauty is originally derived from the doubling of the chromosomes in a hybrid. On the other hand, the present work has shown that diploid gametes occur not infrequently in *C. persicifolia*, and the meeting of two of these may have been the origin of the *Campanula* Telham Beauty.

Chromosome Numbers.

TABLE I.

	No. of plants	No. of chromosomes	
		Haploid	Diploid
<i>C. persicifolia</i>	4	—	16
	4	8	—
Telham Beauty and Selfs	3	—	32
"	4	—	? 32
"	4	—	30-32
"	1	—	29-30
"	1	—	32-33
"	4	16	—

TABLE II

<i>F</i> ₁	No. of plants	No. of chromosomes	
		Haploid	Diploid
Telham Beauty × <i>C. persicifolia</i>	4	—	24
"	5	—	24-5
"	1	—	32
Telham Beauty × " <i>nitida</i> "	7	—	24
"	1	—	? 16
"	1	—	28-30
"	1	—	32
" <i>nitida</i> " × Telham Beauty	1	—	24
"	1	—	32

TABLE III.

F_2	No. of plants	No. of chromosomes	
		Haploid (p.m.c.)	Diploid (root-tips)
From Telham Beauty \times <i>C. persicifolia</i> F_1 plants with 24.5 chromosomes, selfed and intercrossed	1	—	? 16
"	1	8+9	17
"	3	—	18
"	2	—	20-22
"	1	10-13	—
"	1	—	20-25
"	1	—	24
"	1	—	26
"	2	—	24-25
"	2	—	25-26
"	2	—	26-28
"	1	—	27
"	1	—	? 28
"	2	—	27-29
"	1	—	28-29
"	1	—	28-30
"	2	—	29-30
"	1	—	30
"	1	—	30-32

TABLE IV.

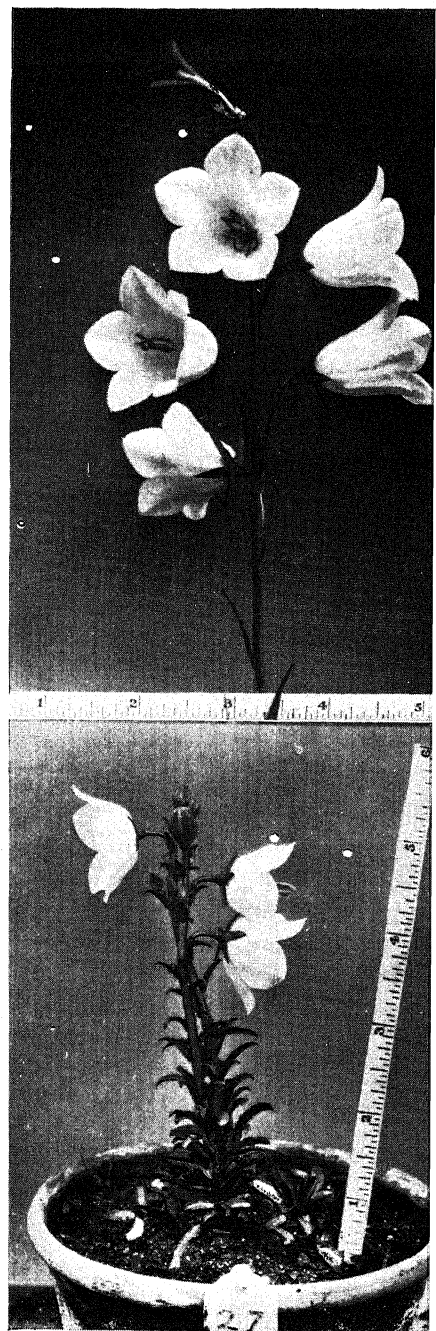
F_3	No. of plants	No. of chromosomes	
		Haploid (p.m.c.)	Diploid (root-tips)
$\frac{13^3}{22}$ Self 24-6 chromosomes	1	? 16	—
"	1	14+14	—
$\frac{13^4 \times 8}{22}$? 22 chromosomes \times 17 chromosomes	3	—	16
"	1	? 8-9	—

TABLE V.

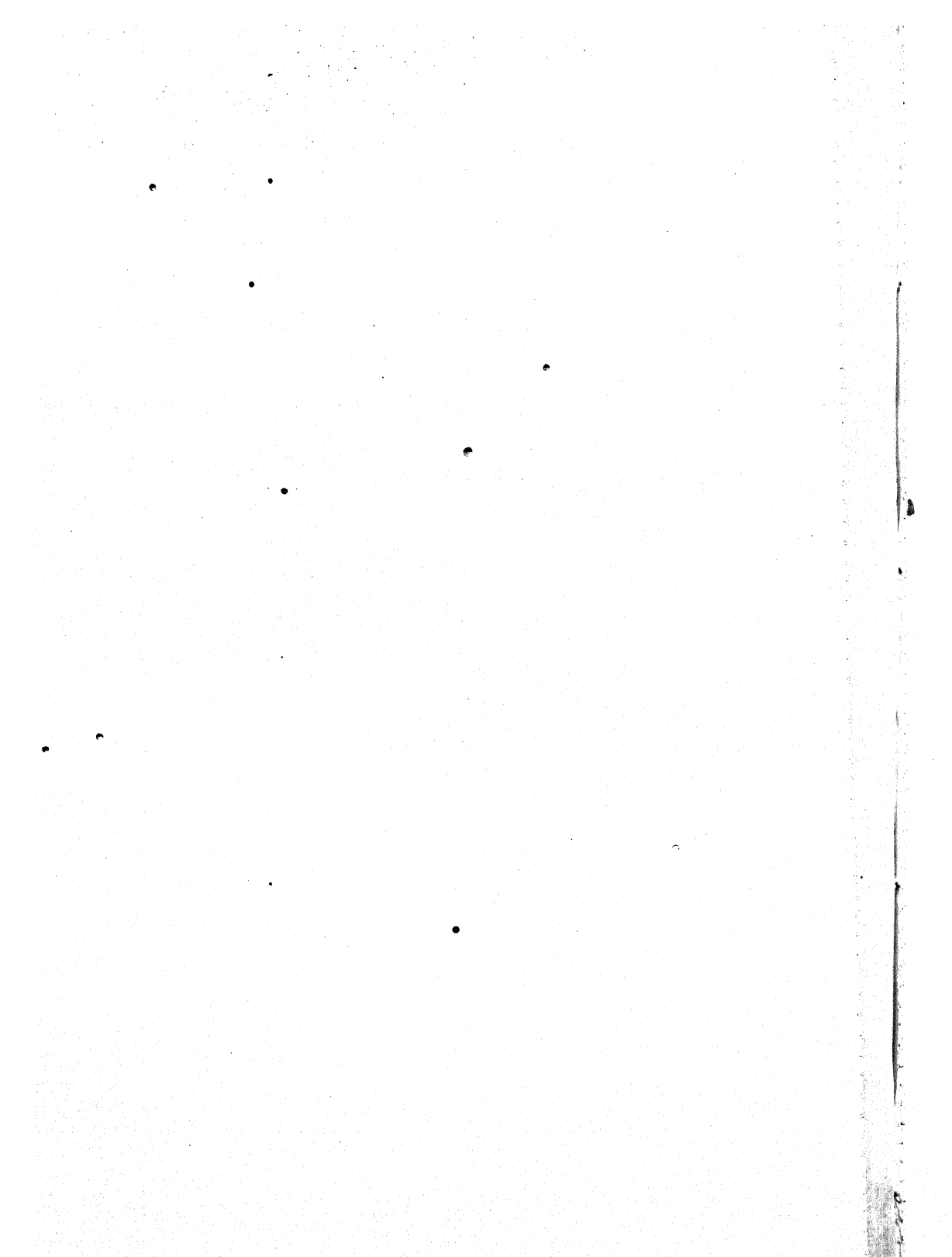
Back crosses	No. of plants	No. of chromosomes	
		Haploid	Diploid
<i>C. persicifolia</i> \times F_1 , 16 \times 24 chromosomes	1	—	? 16
" 16 \times ? 24 "	1	8-9	—
$F_1 \times C. persicifolia$, 24 \times 16 "	2	—	16
" 24 \times 16 "	1	—	18
" 24 \times 16 "	1	—	19
" 24 \times 16 "	1	10 prs + 1	20-22
" 24 \times 16 "	1	—	20-21
$F_2 \times C. persicifolia$, 17 \times 16 "	3	—	16
" 17 \times 16 "	2	? 8	—
" 24-6 \times 16 "	1	—	? 20
" 22 \times 16 "	1	? 10-12	—

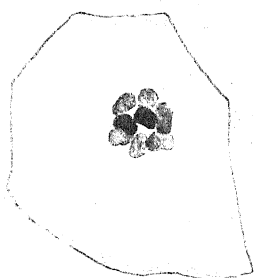


Fig. 1.

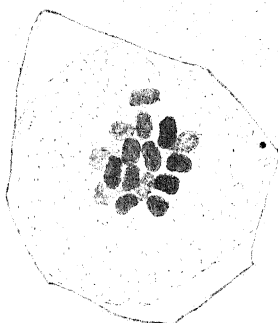


Figs. 2 and 3.





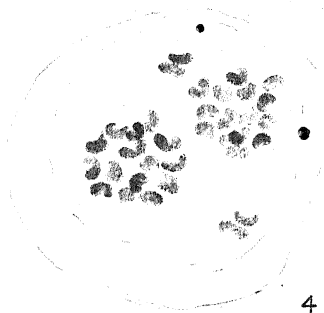
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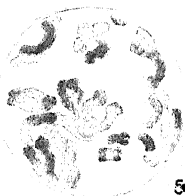
2



3



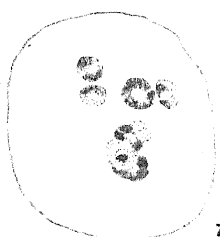
4



5



6



7



8



10



11



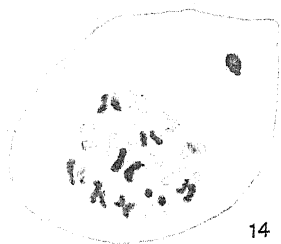
12



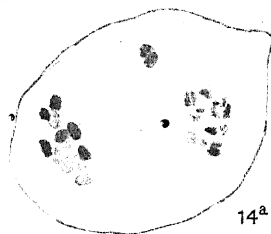
13

5^a

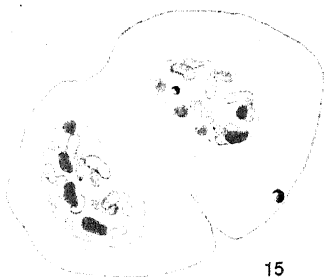
9



14



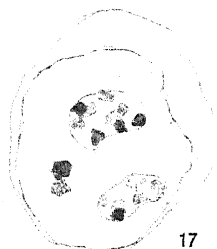
14^a



15



16



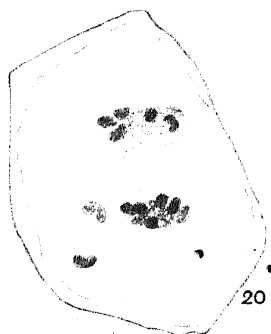
17



18



19

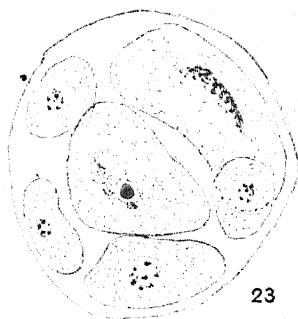


20



21

22



23

TABLE VI.

Back crosses	No. of plants	No. of chromosomes	
		Haploid	Diploid
Telham Beauty $\times F_1$, 32 \times 24 chromosomes	1	—	17
" " 32 \times 24 "	1	—	28-9
" " 32 \times 24 "	1	—	? 30
$F_1 \times$ Telham Beauty, 24 \times 32	1	14-14	—
" " 24 \times 32 "	1	—	? 24
" " 24 \times 32 "	1	—	27-8
" " 24 \times 32 "	1	—	? 30
Telham Beauty $\times F_2$, 32 \times 22	2	—	? 28-30
$F_2 \times$ Telham Beauty, 17 \times 32	1	—	? 30-2
" " 22 \times 32 "	2	—	? 30
" " 22 \times 32 "	1	—	27-8

EXPLANATION OF PLATES XXV—XXVII.

PLATE XXV.

Fig. 1. Telham Beauty.

Fig. 2. *C. persicifolia*.

Fig. 3. "Nitida."

PLATES XXVI AND XXVII.

All material fixed in Zenker's fixative, except Figs. 11, 17 and 22, which were fixed in Flemming.

Figs. 7-10, 14, 18 and 19 drawn from whole cells in pollen smears, all others from material imbedded in paraffin wax and sectioned. Drawings made with camera lucida 12.5 mm. compensating ocular and $\frac{1}{2}$ oil imm. obj. magnification approximately $\times 3000$; where 2 mm. apo. obj. used, $\times 2800$.

Fig. 1. *C. persicifolia*, p.m.c. division, 1st metaphase plate. $\times 3000$.Fig. 2. Telham Beauty, p.m.c. division, 1st metaphase plate. $\times 3000$.Fig. 3. *C. persicifolia*, p.m.c. 1st anaphase plate. $\times 3000$.Fig. 4. Telham Beauty selfed, p.m.c. division, 1st anaphase plate. $\times 3000$.Figs. 5 and 5 a. Telham Beauty, p.m.c. division, diakinesis, one cell, two sections, some chromosomes cut. $\times 3000$.Fig. 6. *C. persicifolia*, p.m.c. division, diakinesis. $\times 3000$.Fig. 7. F_3 , p.m.c. division, diakinesis. $\times 3000$.Fig. 8. *C. persicifolia* $\times F_1$, plant with 8+9 chromosomes, p.m.c. division showing arrangement of chromosomes on spindle. $\times 2800$.Fig. 9. Same plant showing 7 pairs and a string of 3 chromosomes. $\times 2800$.Fig. 10. Same plant showing 2 anaphase plates 8+9 chromosomes. $\times 2800$.Fig. 11. F_1 megaspore mother-cell, 1st division. $\times 2800$.Fig. 12. F_1 p.m.c. division showing univalents and bivalents. $\times 2800$.Fig. 13. F_2 p.m.c. 1st division, 1st anaphase plates 10+9+1. $\times 3000$.Figs. 14 and 14 a. Telham Beauty, irregular division in p.m.c., one cell drawn in 2 planes. $\times 2800$.Fig. 15. $F_1 \times C. persicifolia$, p.m.c. 1st division irregular. $\times 2800$.Fig. 16. $F_1 \times C. persicifolia$, p.m.c. 1st division regular. $\times 2800$.Fig. 17. Telham Beauty selfed interkinesis showing 2 chromosomes thrown out, only part of cell. $\times 3000$.Fig. 18. Triploid $\frac{1}{2} \times$ "nitida," p.m.c. division, univalents, bivalents and trivalents. $\times 2800$.Fig. 19. A sister plant, p.m.c. division, 2nd anaphase plates 13+13 and 11+11. $\times 2800$.Fig. 20. F_2 , p.m.c. 1st anaphase, 2 chromosomes thrown out. $\times 3000$.Fig. 21. F_1 types of trivalents at diakinesis. $\times 3000$.Fig. 22. F_1 type of trivalents on spindle. $\times 3000$.Fig. 23. Telham Beauty selfed, irregular development of pollen grains. $\times 3000$.



SPECIES CROSSING IN THE GENUS *TENEBRIO*.

BY DR A. M. FREDERIKSE.

(With One Plate.)

INTRODUCTION.

IN the course of the extensive investigations carried out by Mr Arendsen Hein of Utrecht on the biology of *Tenebrio* species and the hereditability of variations it was found impossible to cross the various species of *Tenebrio* so that offspring resulted, and it, therefore, became necessary to discover the cause of this. As the sex could already be ascertained in the larvae it was possible to isolate the sexes before puberty. The two sexes were thus separated and after 10 to 12 days in the incubator the species it was desired to cross were brought together, when copulation took place.

♂ *obscurus* × ♀ *molitor*; and reciprocal.

♂ *syriacus* × ♀ *molitor*; and reciprocal.

♂ *obscurus* × ♀ *syriacus*; and reciprocal.

♂ *obscurus* × ♀ *opacus*.

♂ *opacus* × ♀ *molitor*.

The eggs of all these crossings were collected, but out of more than 5000 eggs not a single one developed.

The problems thus confronting Mr Arendsen Hein were threefold and presented themselves as follows:

1. That there was only apparent copulation, the male discharging no spermatozoa into the female of a foreign species.

2. That there was discharge of spermatozoa which did not penetrate the foreign ovum.

3. That fertilisation of the egg took place, but without resulting in development.

OBSERVATIONS.

From my own observations I have ascertained that copulation between the different species of *Tenebrio* has taken place, viz. between

♂ *syriacus* × ♀ *molitor*; ♂ *molitor* × ♀ *obscurus*;

♂ *obscurus* × ♀ *molitor*; ♂ *molitor* × ♀ *syriacus*;

though for lack of time closer investigation was impossible.

I then turned my attention to the first problem quoted above as to whether copulation was only apparent; or, if spermatozoa had been discharged, where they had been deposited. For this purpose a male and a female from the different species under investigation were placed in a separated receptacle and the eggs laid in the buckwheat husks or on the fibres were collected. If these adhered firmly to the husks or fibres, they were fixed for examination and only later separated from them in the alcohol. The following species were thus successfully crossed:

♂ *syriacus* × ♀ *molitor*; ♂ *obscurus* × ♀ *molitor*;
♂ *molitor* × ♀ *syriacus*; ♂ *molitor* × ♀ *obscurus*;
♂ *obscurus* × ♀ *syriacus*.

In all these cases sperm could repeatedly be traced in the spermatheca of the female. This I first ascertained by firing and examining the sex organs in sections of some of the females. As this method, however, required much time I took to preparing the sex organs and examining them alive under the microscope in Ringer's solution, which method proved exceedingly suitable. By these means I could establish that not only was sperm deposited in the proper place, but that it was alive, which fact could be proved by the rhythmic movements it made, these being clearly perceptible. It is true that in comparative examinations with *Ten. mol.* × *Ten. mol.*, I got the impression that fewer spermatozoa were deposited in the spermatheca in crossing species than in the fertilisation of *Ten. mol.* × *Ten. mol.* Also in crossing different species sperm was not regularly found in the spermatheca, and many females which had lived with males of another species for a considerable time had received no sperm. In a very few instances sperm was found only in the vagina. Thus side by side with females in the species-crosses where fertilisation had exactly the same result as in the case of *Ten. mol.* × *Ten. mol.*, many females being found having little or no sperm in the spermatheca.

The second problem, whether the spermatozoa penetrate the eggs, proved to be more difficult for purely technical reasons. Already in former researches I found that to fix and make microscopical preparations of these eggs, which contain much yolk, required great care. As regards the technical process followed in this investigation I must refer to what has already been stated in connection with the examination of the unfertilised eggs of *Tenebrio* (4, 1924). In this case, too, the difficulty was to obtain sufficiently thin sections in series, which is absolutely necessary to be able to see the two ♂ and ♀ pronuclei in the very earliest

stage. The whole of the egg must be laid out in complete sections under the microscope and in some cases this was successfully done. But the earliest stage is seldom met with, which leads to the conclusion that it must be gone through very quickly. Thus we see in Fig. 2 in one section the egg nucleus engaged in excluding the second polar body, and three sections further a nucleus in a mass of protoplasm in the middle of the yolkgrains (Fig. 2 a); since no nuclei are to be met with further in the egg, we may assume that this is the male pronucleus. I have also in a few instances found a structure in the outermost layer of the egg which I take to be the head of the spermatozoon which had penetrated into the egg.

It is thus proved that the spermatozoa penetrate the ovum and that the egg is fertilised.

There remains the problem why no offspring resulted from the eggs collected from the species-crosses. Not only did Mr Arendsen Hein never see development from any of the large number of eggs he collected, but in my own investigations too the results were negative, notwithstanding the most careful supervision, which makes it absolutely certain that among these eggs there were no *molitor* ♀ fertilised by *molitor* ♂ or *obscura* ♀ by *obscura* ♂, etc.

I now began the cytological examination of these eggs in order to find some explanation for the non-development of the fertilised eggs. By way of introduction to this examination I had beforehand investigated the non-fertilised eggs of *Ten. mol.* (4, 1924) to ascertain the course they followed. It then appeared that in the non-fertilised eggs also, a certain development, up to an imperfect blastomere stage, had taken place, showing that not each and every development of the cross-fertilised eggs was to be ascribed to cross-fertilisation.

Lecaillon had already obtained the same result with the non-fertilised egg of hen and peacock (1910).

It now appeared that the cross-fertilised eggs took a much more regular course than the non-fertilised eggs. Here in most cases there was no imperfect blastomere formation, but all the transitions from one stage of development to another were to be found, ranging from cases in which the cells varied to some extent in size up to those in which the whole circumference of the egg is covered with exquisite and sometimes almost exactly similar and regular blastoderm cells, which often scarcely differed in any respect from the eggs of the *Ten. mol.* × *mol.*

It is true that, side by side with these, irregular eggs are also met with, but this can also happen with the eggs of *Ten. mol.* × *mol.*, where

the eggs do not all appear to receive the spermatozoa either; this is just as likely to occur in the species-crosses, though perhaps on a larger scale, seeing that amongst these the number of eggs that degenerate at an early stage is much greater, but I cannot be quite certain of that. We find, however, that eggs which look normal when slightly magnified, show irregularities in the cells when highly magnified, principally in the mitosis in the blastomeres. These irregularities do not occur in all the cells and the stages at which they begin to appear may also vary considerably. Moreover, the extent of the deviations from the normal, *i.e.*, the percentage of the cells which show such deviations in the developing eggs, varies very much.

If we compare the position of the chromosomes in the mitosis of the eggs of crossed species with those of the eggs of *mol. × mol.*, we notice that while the placing of the chromosomes in the eggs of the *mol. × mol.* is very regular, really forming one plane (seen from the side of the section as one line), in the eggs of species-crosses there is generally nothing of the sort. The chromosomes are more scattered, at varying distances from the poles.

In the course of the mitosis, the chromosomes in the eggs of species-crosses also act very differently and move at varying rates towards the two poles, so that they get further and further apart; so much so that in extreme cases (and these are really not so very unusual) certain chromosomes get so far behind that they are not taken up into the new nucleus at all. This means a loss of chromosomes for the new nucleus about to be formed (Figs. 3 and 4).

If this is repeated several times—and it is self-evident that the force once working abnormally will continue so to work in the following mitosis—then it means a very serious loss for the cell.

We are thus reminded of the researches of Boveri into multipolar mitosis in dispermic sea-urchin eggs (1902–7), where through the deviation of the distribution of the chromosomes, an abnormal development took place and—in the case of too great a shortage of normal chromosomes—the embryo either died off or developed abnormally.

In this recurring loss of chromosomes we may presume to have found the cause of the non-development of the eggs under discussion. The fate of the chromosomes which come to be on the outside of the nucleus, in the way described above, is most probably to be re-absorbed into the protoplasm. This conclusion is formed because all the stages can be seen, from chromosomes plainly lying outside the mitotic figure up to dark spots in the protoplasm outside, the resting nucleus hardly showing

chromatic staining. Very occasionally a few smaller nuclei succeed in forming themselves near the real nucleus (*Karyomeren, Teilkerne*).

It is here important to point out that the size of the blastomeres and particularly of the nuclei of these cells differs greatly. This is the natural consequence of the very unequal quantities of chromatin with which, in the long run, the nuclei of the blastomere cells are provided, as it may happen that into one nucleus not one single chromosome is passed, and into another nucleus several (cf. Figs. 5 and 5a). This phenomenon also was observed by Boveri in the investigations quoted above, where the nucleus of various regions of the developing embryo had received varying quantities of chromatin and were consequently of different sizes. We cannot point to any difference in the size of the chromosomes, such as Harrison, Doncaster, Federley, Moenkhaus, and others have described in their researches in hybridisation, as here no great difference can be perceived between the chromosomes of the parents.

Another deviation from the normal which is met with in the later stages and in increasing measure as the egg develops, is the change in the shape of the chromosomes. While in the earlier stage the shape of the chromosomes was pointed as in the eggs of *Ten. mol.* \times *Ten. mol.*, the later stages of cross-breeding show large bar-shaped, comma- or S-shaped chromosomes (Figs. 6, 7, 8, 9). The existence of these chromosomes is probably due to the conjunction of the original small forms, as the total number has become smaller, and, from the construction of the chromosomes too in certain clear cases, we are justified in concluding that this conjunction has taken place, since reproduced schematically it is as follows: $\wedge\wedge\wedge$

The number of chromosomes in the equatorial plane can be ascertained, but there is no object in doing so, since the shape, and consequently the number, as I have just stated, is variable. Side by side with cases in which the chromosomes are reduced in number, owing presumably to conjunction, nuclei are found where there are apparently the normal pointed chromosomes, but too numerous (Fig. 10). Here, probably, certain chromosomes have fallen apart, forming small ones.

One peculiarity, which is often observed in the resting nucleus of the developing eggs in these crosses can be explained as a direct consequence of the nucleus being constructed of two component parts which differ too much from each other. For instance, very often the chromatin can be seen heaped up in two separate masses in the nucleus (Figs. 11 and 12). This can be seen even more plainly when there are two separate karyomeres (Figs. 13 and 14). These forms are repeatedly met with, sometimes

in many adjacent cells. In the indirect division too it can be seen that the chromosomes remain divided in two separate groups and that the division of these groups takes its course each independent of the other (Figs. 15-21 incl.), which is strongly reminiscent of the mitosis in the developing *Cyclops* eggs described by V. Haecker (1895).

Several varieties may be seen of these double nuclei varying from two separate nuclei lying side by side or two chromatin heaps in a single nucleus up to dumb-bell-shaped ones (Fig. 22). At this point it must be emphasised that all these shapes of nuclei are very often met with, whilst they are scarcely ever found in the *mol. × mol.* fertilisation—certainly not more frequently than in any other ordinary tissue. In the stages after blastomere formation, pycnosis very often sets in more and more markedly; further development is thus brought to a standstill and the embryo dies, although the development may be fairly advanced before this takes place.

It will suffice now if I point out—with the aid of a few illustrations (Figs. 26, 27 and 29)—the stages of development—probably normal—which are sometimes gone through in such cases.

If, for instance, we look at Fig. 27, the most advanced stage of development I met with in my sections, it can be seen that a considerably advanced stage may be reached. It is a great pity that the section is not complete, but it is distinct enough for us to see how it agrees with the illustrations given by other writers who give figures of this stage. If this diagram is compared with Fig. 3 of N. de Sélys Longchamps (1904) we see that the embryos from which these figures were made cannot differ greatly in age. De Sélys Longchamps states that the age of the embryo in his Fig. 3 was seven days. We even see what this writer calls “l’appendice du premier segment abdominal” represented in the section I have reproduced here.

Although it has been shown in the preceding pages that the development of the embryo may be fairly normal, it must be emphasised that, side by side with the normal, much irregular development occurs, and this already in the blastomere stage. We see, for instance, a strong irregular growth of the cells on the circumference of the egg where they form a large irregular heap (Fig. 30), or again the cells do not occupy the whole of the circumference, but only a small portion here and there (Fig. 28). It is interesting to observe the agreement between the course of development and the deviations in the mitosis spoken of in the preceding pages.

Owing to the entirely fortuitous emission of various chromosomes

the course of development varies greatly. Taking into account the altered shape of the chromosomes in the hybrids various chromosome-combinations may result when a "*Sammel-chromosom*" is emitted. Development may thus come to a standstill at a very early or a very late period, there may be abnormal irregular growths with embryo-formation, etc., and it would, therefore, not be surprising if it were found possible from a very large number of crossings between various species of *Tenebrio*, to get larvae or even imagines. Comparing the development of the cross-fertilised egg with that of the non-fertilised egg of *Tenebrio* as described in a former article of mine (1924), it is found that, although in this case too large a proportion of the eggs degenerate early, yet the greater proportion reach a fairly complete and fairly regular blastomere formation. A small proportion get as far as embryo formation before degenerating. No distinct difference can be traced between different crossings.

DISCUSSION.

On a survey of similar researches recorded in biological literature it is seen that disturbances of the mitosis of hybrids set in at very different periods of development.

Arranged according to the time at which such disturbances set in, a systematically graduated series may be drawn up which runs parallel with the closer or more distant kinship of the species crossed. At one end of this series appear species differing so little from each other that under normal circumstances they have a normal and fertile offspring. Cannon (1903) states that in the crossing of *Gossypium Barbadosense* \times *herbaceum*, as a rule normal mitoses are met with, but that in material fixed late in the year, irregular mitoses appeared just as in the case of completely sterile hybrids. Then come the crossings which always for the greater part result in sterile hybrids, or those which have exclusively sterile offspring. This calls to mind the experiments of Guyot (1902-3) with pigeons, Geoffrey Smith (1914) with pigeons and pheasants, Poll (1911) with ducks, Woodsalek's researches on the mule, H. Federley's crossings of Lepidoptera (1914-15), and the researches of Harrison and Doncaster (1914). All the above-mentioned show deviations in the chromosome pairing, which appears either in the syndesis, or in the meiosis, or in both. To this group belong, in the province of botany, the crossings of *Hieracium* and *Drosera* described by Rosenberg, the experiments with roses carried out by Täckholm, Blackburn and Harrison and many others. For a survey of the action of chromosomes in hybrids attention

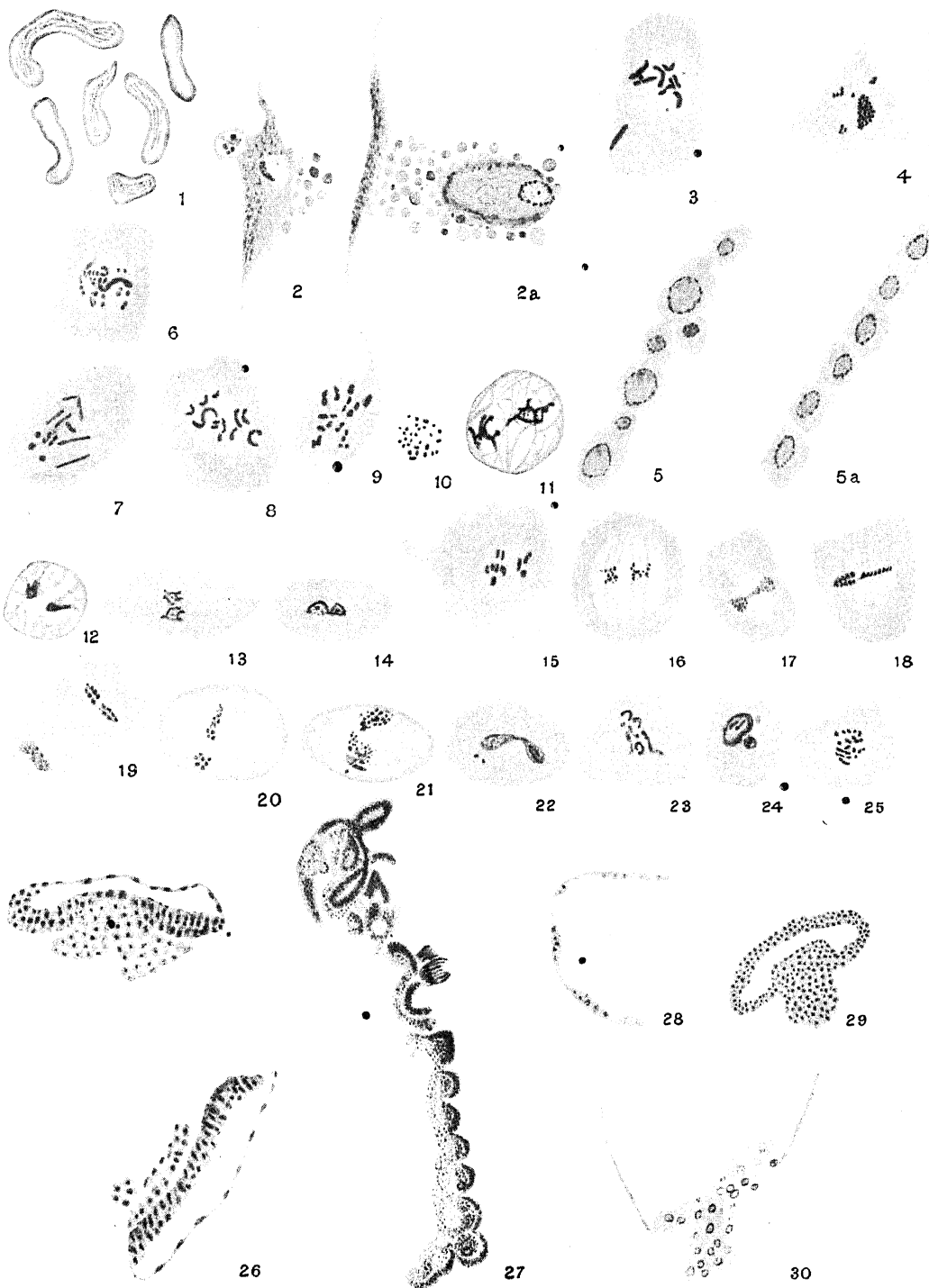
is drawn to Wilson, *The Cell*, pp. 841-853 (1925), and Tischler, *Allgemeine Pflanzen Karyologie*, pp. 430-452.

On the other hand, for example, it must be pointed out that at the opposite end of the series there is the case related by Godlevski, of the fertilisation of a sea-urchin egg by the sperm of an annelid or mollusc. Here there is no question of crossing, as in the heterogeneous fertilisation, the sperm only plays the part of activator of the egg cell, whilst its chromosomes play no part in the further development. When the difference between egg and sperm-cell is not quite so great, then what occurs is similar to what Baltzer (1909) describes in the crossing of *Paracentrotus* \times *Sphaerechinus*, where nearly all the male chromosomes were unable to adapt themselves to the female egg-plasm and were ejected. The peculiarity of the case is that in reciprocal crosses a similar occurrence did not take place. Moenkhaus's examination (1904) into the development of crosses between *Fundulus* and *Menidia* is very interesting on account of the various shapes of the chromosomes. In these cases development ceases already during the segmentation or gastrulation, and abnormal developments are numerous.

Comparing the present researches with this bird's-eye view of hybrid cytology, it becomes evident that in our case development never reaches the stage at which the larvae leave the egg, and that development may cease or degeneration set in at any of the stages at which such is possible, which is not surprising considering the very irregular loss of chromosomes in the mitosis.

A similar loss of chromosomes in the mitosis is described by Baltzer. But there the character of the chromosome-loss is very different, since the 16 or 17 chromosomes which are unable to adapt themselves to the new plasma are all emitted at the same time at the first division. In *Chromosomestudien an Mischlingen* (1914-15), H. Federley describes in the first meiotic division of *Dicr. ermina* \times *vinula*, a phenomenon similar to the one I have described above, namely the union of several chromosomes, which explains the altered shape of many of them and the frequent decrease in numbers. Federley takes it to be a chromatolytic phenomenon (Fig. 8 in the paper just cited). Doncaster and Gray (1913), too, describe this cohesion of chromosomes in crossing *Echinus acutus* \times *esculentus*.

The phenomenon I have observed, that the figures in the mitosis of hybrids are not so regular as those of the parents, that the equatorial plate, as seen from the side, covers a greater surface (thus giving an effect of irregularity), is confirmed by most investigators (see fig. 10 in Federley's article just quoted, compared with his figs. 2, 3, 5, 6), which





shows in one hybrid the same deviation first occurring in the meiosis, which in another already occurs in the embryo-development. A cross which took a course similar to the one here described by me is *Ech. miliaris* ♀ × *esculentus* ♂, and *miliaris* ♀ × *acutus* ♂, which Doncaster and Gray have described. Here, too, side by side with very regular mitoses, there were mitoses where chromosomes were ejected.

CONCLUSIONS.

In species-crosses in the genus *Tenebrio* fertilisation of the egg does take place.

Owing to the deviations from the normal in mitosis the embryo does not reach the larval stage.

Embryonic development differs greatly in abnormality and in the stages it reaches.

This greatly differing development, taken in conjunction with the greatly varying emission of chromosomes, may be taken as supporting the theory of chromosome-heredity.

EXPLANATION OF PLATE XXVIII.

All the numbers of oculars and objectives refer to the Leitz microscope.

Fig. 1. Section of spermatheca of *T. obscurus* fertilised by *T. molitor*.

The cut tube of this organ occurred six times in the part of the section here sketched, and the four central of these contained sperm; all the other cells and nuclei have not been drawn. Fixing Bouin. Colouring haematox. orange G.

Fig. 2. Egg of *T. syriacus* fertilised by *T. mol.* Emission of polar body. Formalin. Haematox. Eosin. Oc. 3; obj. 8.

Fig. 2 a. ♂ pronucleus: same egg.

Fig. 3. Blastomeres from crossing *T. obsc.* ♂ × *T. syriacus* ♀. Emission and retention of chromosomes. Sublim. formal. Haematox. Bord. red.

Fig. 4. Ditto.

Fig. 5. Irregular size of cells in cross *T. obsc.* ♂ × *T. mol.* ♀.

Fig. 5 a. Regular cells in *T. mol.* × *mol.*

Fig. 6. Cell from cross *T. syr.* ♂ × *T. mol.* ♀. S-shaped chromosome.

Fig. 7. Equatorial plate from cross *T. syr.* ♂ × *T. mol.* ♀. Oc. 4; ol. imm. $\frac{1}{12}$.

Fig. 8. Greatly altered shape of chromosome in cross *T. obsc.* ♂ × *T. syr.* ♀.

Fig. 9. Abnormal chromosomes (in pairs!). Cross *T. syr.* ♂ × *mol.* ♀. Oc. 4; imm. $\frac{1}{12}$.

Fig. 10. Equatorial plate. Cross *T. obsc.* ♂ × *syr.* ♀. Number of chromosomes too many. Oc. 5; imm. $\frac{1}{12}$.

Fig. 11. Chromatin in two groups. Cross *T. syr.* ♂ × *mol.* ♀. Oc. 5; imm. $\frac{1}{12}$.

Fig. 12. Ditto. Sublim. formalin. Oc. 3; obj. 8.

Fig. 13. Double nucleus. Cross *T. syr.* ♂ × *mol.* ♀. Oc. 3; imm. $\frac{1}{12}$.

Fig. 14. Ditto.

Fig. 15. Chromosomes in two groups. *T. syr.* ♂ × *mol.* ♀. Oc. 3; imm. $\frac{1}{12}$.

Figs. 16-19. Ditto.

Figs. 20-21. Ditto. Two nuclei in section lying near each other.

Fig. 22. Dumb-bell-shaped nuclei *T. mol.* ♂ × *syr.* ♀.

Fig. 23. Abnormal chromosome shapes. *T. syr.* × *mol.* Oc. 4; imm. $\frac{1}{2}$.

Fig. 24. Supernumerary nuclei. *T. mol.* ♂ × *syr.* ♀. Oc. 3; obj. 8.

Fig. 25. Equatorial plate *T. obsc.* ♂ × *obsc.* ♀.

Fig. 26. Developed embryo from cross *T. mol.* ♂ × *obsc.* ♀. Rest of egg omitted. Oc. 2; obj. 4.

Fig. 27. Most advanced stage met with. Section incomplete. *T. obsc.* ♂ × *syr.* ♀.

Fig. 28. Irregular blastomere formation. *T. syr.* ♂ × *mol.* ♀.

Fig. 29. Section embryo. *T. syr.* ♂ × *T. mol.* ♀. Oc. 2; obj. 3.

Fig. 30. Growth in a single place on circumference of egg. *T. obsc.* ♂ × *syr.* ♀.

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GENETIC STUDIES IN *BRASSICA OLERACEA*

By M. S. PEASE, M.A.

(With Three Plates and Seventeen Tables.)

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INTRODUCTORY.

THE striking diversity of the cultivated varieties of *Brassica oleracea* cannot fail to excite the interest of the geneticist. To the experimenter, however, this plant, as material for genetic analysis, presents a number of drawbacks; some are obvious from the start, and others become evident in the course of breeding. To the first class belong the size of the plant (each individual occupies nearly a square yard of land), its biennial period of growth, and the liability of the plants to damage by frost, hares, and rot during the winter months. To the second class belong the frequent partial or complete self sterility, the awkward problem of loss of vigour resulting from continued inbreeding, and the fact that the flowers do not naturally pollinate themselves. The most serious difficulty about this species as material for exact genetic analysis is that the principal characters which come in question, the "Bulb" of the Kohl Rabi, the "Heart" of the cabbage, the Curly foliage of Scotch Kale, and the deformed inflorescence of the cauliflower, are not only

characters which, on crossing, give a continuous series from one extreme of expression to the other, but are also characters which, in practice, are incapable of exact measurement. But in spite of these obstacles the material offers two great attractions to the plant breeder, one of practical, the other of theoretical significance. The practical attraction is this: *Brassica oleracea* has already provided some of our most important vegetable and field crops. Might it not be possible to combine by crossing the virtues of some of the existing types, or perhaps even to induce *oleracea* (apparently endowed with an unusual proclivity to vary) to bring forth yet another variant useful in some novel way? The point of theoretical interest turns on the fact that *B. oleracea* has a fairly small number of chromosomes, namely nine, and that therefore the expectation of finding interesting linkages is considerable. Let it be said at once that so far no "practical" results have emerged from the experiments. But, on the other hand, the linkages which have so far been brought to light, show every promise of being uncommonly interesting, though the task of unravelling them at present seems formidable.

One of the results of the difficulties which face the experimenter with this plant is that until recently very little has been published on the genetics of *B. oleracea*. For a long time the only recorded experiments were those of the late A. W. Sutton, who made crosses between Cabbage, Kohl Rabi and Thousand-headed Kale as long ago as 1907. He raised a considerable F_2 in each case and showed clearly that segregation took place; but the account has suffered from the author's unnecessary loyalty to the now all too familiar 3 to 1 ratio, at that time the indispensable hall mark of orthodox Mendelism. Last year (1924) Kristofferson published a long account, profusely illustrated, of the crosses:

Cabbage by Curly Kale,
Cabbage by Brussels Sprouts,
Cabbage by Broccoli
and Brussels Sprouts by Broccoli.

This conveys an admirable impression of the general character of the F_2 segregation in the *oleracea* crosses. Extracted pure parental types are rare, and at first glance the cultures present the appearance of an almost uniform intermediate type. The author has worked with prodigious numbers: many of his F_2 cultures exceed 10,000 plants. But unfortunately in counting this mass of material, he has recorded, not the plants, but only the characters. By so doing he had failed to bring to light the

linkage relationships, which I hope to show are by far the most interesting thing about *B. oleracea*. Kristofferson's numbers, however, definitely shatter Sutton's suggestion of single factors and show with certainty that many factors are involved, though Kristofferson himself refrains from making a guess at their number; nor does he attempt to define any of them except the single factor which, in most cases, causes the difference between the *oleracea* types with purple foliage and those with green.

By far the most interesting paper on *B. oleracea* is that of Malinowski, published in the *Mémoires de l'Institut Génétique de l'École supérieure d'Agriculture* in Warsaw as far back as 1921, but which has only recently come to the present writer's notice. In a cross between Cabbage and Curly Kale, Malinowski has brought to light an instance of a peculiar form of linkage which my own experiments have led me to believe to be fairly common in *B. oleracea*. Malinowski raised an F_2 of 584 plants from this cross, and from his counts deduces that the heart of the cabbage is dependent on three strictly polymeric factors, A , B , C , and that similarly the curly character of the Kale foliage depends on three polymeric factors X , Y , Z . He finds, in recording his F_2 into seven classes, a rigid association between heart and smooth leaf on the one hand, and curliness and absence of heart on the other, not only in the extracted parent types, but also throughout all the five intermediate grades of each character. In other words, a correlation table with respect to these two characters is a straight line without any deviation into lateral classes (Table XIV, p. 381). From this it follows that there must be very intimate linkage between A and x , B and y , and C and z . It must be confessed, however, that the diagram on p. 7 of Malinowski's text, which purports to illustrate in terms of chromosomes this scheme of linkage, is entirely obscure; unless, indeed, the author has intended to introduce a modification of Morgan's theory. But apart from this, it is clear that the notion of three pairs of closely-linked factors is a possible explanation of the case. But it is far too elaborate a structure to base on the evidence of a solitary F_2 culture; it requires confirmation. And in view of the astonishingly good fit between the numbers of plants in the intermediate grades found, and those expected on Malinowski's theory, there should have been very little difficulty in picking out a number of homozygous intermediate plants of the classes demanded by the hypothesis.

My own experiments have so far brought to light four such cases of linkage in *B. oleracea*, namely, between the "Bulb" of the Kohl Rabi

and the "Heart" of the Cabbage, between the "Bulb" and the Curly foliage of the Scotch Kale, between the deformed inflorescence of the Broccoli and the "Heart" of the Cabbage, and between the latter and the Curly Kale (Malinowski's case). The experiments were started in 1912, the first crosses being made under glass in 1913; the F_1 plants were grown out of doors in the same year and the F_2 seed was harvested just before the outbreak of the war. Both F_1 and F_2 seed was sown in 1919 and 1920 and much of it germinated well; but the seed of the parent lines, obtained by self pollination, did not survive the five years' storage. Consequently new parent lines have had to be introduced into the post-war experiments; but so far the results have afforded no reason for supposing that this new material is not genetically comparable with that of the original experiments.

The F_2 cultures in 1919 showed at once that "Heart," and "Bulb," and the curliness of foliage, and the Broccoli inflorescence were all complicated characters, dependent on more than one factor each. Any two of these characters crossed together gave an F_2 which showed this peculiar sort of linkage, not only in the extracted parent types, but also throughout all the intermediate classes. As we have seen in discussing Malinowski's case, where on crossing, two characters, each dependent on its own set of polymeric factors, show "association" in F_2 , there exists the possibility of multiple linkage. To the present writer the interest seems to lie, not so much in this possibility (which is, after all, fairly obvious), but in the definite determination by experimental breeding of the exact scheme of multiple linkage which, in these *oleracea* crosses controls the distribution of types. It has been with this aim continually in view that the experiments have been carried on ever since the 1919 F_2 cultures indicated the nature of the problem to be attacked. Clearly in order to establish beyond question a case of multiple linkage, it is necessary to determine the number of multiple factors involved and to define the action of each. If possible, as a preliminary, the mode of inheritance of a number of independent mono-factorial characters should be established, in order to build up a chromosome framework into which the various multiple factors could subsequently be fitted. The present communication, therefore, is an account of some preliminary experiments: it will be more profitable to defer discussion of the general problem of multiple linkage until experiment has clearly determined the factors involved in such a case.

THE INHERITANCE OF "HEARTING" IN THE CABBAGE.

In the ordinary Cabbage, the leaves of the terminal shoot are closely folded one over the other, forming the familiar dense head or heart (Plate XXIX, fig. 1). In shape this may be pointed (Ox-heart type) or flat (Drumhead type), and as regards size, a good exhibition specimen may be a foot or more across, less fortunate ones perhaps only a few inches. In the Brussels Sprouts, the same sort of deformity occurs, not in the terminal shoot, but in the laterals; a good marketable specimen should have all the lateral buds transformed into hard buttons, which are, to all appearances, miniature cabbages. It is noteworthy that the two forms of this peculiar monstrosity are determined by independent Mendelian factors and that in the commonly cultivated types, both forms do not appear on the same plant. It is proposed to deal with the cabbage type first.

It is important to mention that in experiments with cabbages, regard must always be had to the effect of seasonal and other environmental conditions on the heart. Generally speaking, it is only well-grown plants which produce good hearts; plants which have suffered starvation due to crowding, drought, shade, or late planting usually fail to form sound hearts. On the other hand, seedlings which have been forced under glass and planted out unduly early are apt to "bolt," especially if there is warm weather in early autumn. It is important, therefore, to keep the cultural conditions, in so far as the weather permits, uniform from year to year. Attention, too, must be paid to the time of recording the plants. For the most part this has been done in November and December, and to a less extent in January. But if the winter has been mild the later records cease to be comparable, not so much as regards the genuine firm hearts, but rather as regards the looser grades of hearting, in which the "Heart" becomes more quickly disintegrated by the growth of the flowering shoot. No experiments have been tried with spring cabbages; in all the experiments described below, the seeds have been sown in March, and the plants put out into the field in May whenever the weather permitted.

The F_1 from the cross Cabbage by Kale, Kohl Rabi, or by any other non-hearting *oleracea* (other than Brussels Sprouts) is a plant with no more than a faint indication of hearting. The leaves of the rosette are curved inwards, but they do not at any stage form a closed head (Plate XXIX, figs. 3-6).

In the F_2 generation, both the parent forms were recovered, but the

vast majority of plants as regards hearting formed a continuous range of types from one extreme to the other (Plate XXX, figs. 1-8). I believe, however, that two factors are sufficient to account for the F_2 results, and it will be as well to define these at once. Both these factors, N_1 and N_2 , are ordinarily present homozygously in the Kale type of *oleracea*. When both are absent homozygously the familiar hard cabbage heart is formed. If to this one dose of either N_1 or N_2 is added, the class "Slight heart" is produced (Plate XXX, figs. 2 and 5). In this type, the heart instead of being firm, is loose; the leaves actually fold over one another, but no head is formed sufficiently compact to prevent the free growth of the flowering shoot, as is the case with genuine marketable "Hearts." When either factor is present homozygously or when both are present in the heterozygous condition, the F_1 type is produced: this is entered in the records as "Very slight heart" (Plate XXX, figs. 3 and 6). When three or four doses of N are present, the parent form "No heart" results (Plate XXX, figs. 7 and 8). The following scheme sets out this hypothesis more clearly:

$$\begin{array}{lcl}
 n_1 n_1 n_2 n_2 & = & \text{"Heart."} \\
 \left. \begin{array}{l} n_1 N_1 n_2 n_2 \\ n_1 n_1 N_2 n_2 \end{array} \right\} & = & \text{"Slight heart."} \\
 \left. \begin{array}{l} n_1 n_1 N_2 N_2 \\ N_1 N_1 n_2 n_2 \\ N_1 n_1 N_2 n_2 \end{array} \right\} & = & \text{"Very slight heart."} \\
 \left. \begin{array}{l} N_1 N_1 N_2 n_2 \\ n_1 N_1 N_2 N_2 \\ N_1 N_1 N_2 N_2 \end{array} \right\} & = & \text{"No heart."}
 \end{array}$$

From this it follows that the expected distribution of these types in F_2 is in the ratio 1 : 4 : 6 : 5. It should be stated that the segregation into these four classes is not in every case sharp, the variation is more or less continuous. The "Hearts," however, can be picked out with tolerable certainty; but between the "Slight hearts" and the "Very slight hearts," and between the latter and the "No hearts" there are usually several plants on the doubtful borderland between the classes. With this qualification, therefore, the following table gives the distribution of types in the F_2 cultures found by experiment, the figures in italics being in each case the expectation on hypothesis outlined above.

On the whole the agreement between observation and theory is tolerable; there is some deviation from expectation in the intermediate

TABLE I.

 F_2 . Cabbage (heart) \times "No heart" types. $n_1n_1n_2n_2 \times N_1N_1N_2N_2$.

Cross	Reference*	Year	Heart	Slight heart	Very slight heart	No heart	Total
Kohl Rabi \times Cabbage	Bk. 5, p. 18	1920	43 (41.5)	167 (165.5)	271 (248)	179 (207)	661
Cabbage \times Curly Kale	Bk. 6, p. 13	1921	10 (4)	14 (17)	29 (26)	17 (22)	70
Kale \times Savoy Cabbage	Bk. 6, p. 115	1922	7 (4.5)	23 (18)	23 (27)	19 (22.5)	72
Kohl Rabi \times Cabbage	Bk. 7, p. 168	1924	2 (1.5)	3 (6.5)	10 (10)	11 (8)	26
Savoy \times Kohl Rabi	Bk. 8, p. 6	1924	8 (13)	36 (52)	81 (78)	83 (65)	208
Total	—	—	70 (65)	243 (258)	414 (388)	309 (323)	1036

* The reference is in every case to the field note books in which the plants were recorded at the time of counting.

classes, but the number of extracted "Hearts" points unmistakably to a simple two-factor relationship. Malinowski found only nine hearts out of an F_2 of 584 plants, which is exactly the expectation on a 1 : 63 ratio; and hence he concluded that three factors were at work. Kristofferson found 68 in 6300 and concludes that "a determination of the number of factors involved is not possible."

In any case of this sort, and more particularly where the material is subject to seasonal variation, a single F_2 culture, however large, is insufficient to clinch the matter. The most definite test, in the case of multiple factors, is the Back cross. If the two-factor hypothesis is correct, then the Back cross

$$F_1 \times \text{Cabbage} \\ N_1n_1N_2n_2 \times n_1n_1n_2n_2$$

should give "Hearts," "Slight hearts," and "Very slight hearts" in the ratio 1 : 2 : 1. The actual figures found were 25 : 53 : 21 (Bk. 7, p. 181)¹. On Malinowski's hypothesis we should expect only one heart in eight, instead of one in four found by experiment. This test therefore gives support to the simpler two-factor theory.

Furthermore the F_1 crossed back to the Kale parent, on the two-factor hypothesis would be

$$F_1 \times \text{"No heart"} \\ N_1n_1N_2n_2 \times N_1N_1N_2N_2$$

which clearly would give the ratio "No heart" : "Very slight heart" as 3 : 1. The actual figures found by experiment in two such cases were as follows:

¹ A similar Back cross this year (1925) gave 41 : 88 : 51 (Bk. 8, p. 171).

TABLE II.

Cross back. $F_1 \times$ Kale type.

Cross	Reference	Year	No heart	Very slight heart	Total
F_1 (Savoy \times Kohl Rabi) \times Kohl Rabi	Bk. 7, p. 172	1924	101 (90)	18 (30)	119
F_1 (Savoy \times Curly Kale) \times Curly Kale	Bk. 8, p. 44	1924	176 (182)	67 (61)	243
Total	—	—	277 (271½)	85 (90½)	362

In this case again Malinowski's hypothesis would require only 12.5 per cent. to be in the class "No heart," as against 76.5 per cent. found by experiment, 75 per cent. being the expectation on the simpler two-factor theory put forward in this paper.

Another obvious test in such a case is to examine the behaviour of the various F_2 types when grown on to the next generation by selfing. On the two-factor theory we should expect that:

- (1) "Hearts" should always breed true.
- (2) "Slight Hearts" should never breed true and never give the complete range of types.
- (3) "Very slight hearts" should either breed true [$N_1N_1n_2n_2$] or give the complete range [$N_1n_1N_2n_2$] like the F_1 plants.
- (4) "No hearts" should either breed true ($N_1N_1N_2N_2$) or should segregate into "Very slight hearts" and "No hearts" in the ratio 1 : 3 ($N_1N_1N_2n_2$).

The results of such a breeding analysis are given in Table III, and so far as they go, they clearly support the two-factor theory. There are a few discrepancies, several plants being recorded with hearting of a higher or lower grade than would be expected; but with a character so very easily influenced by external conditions, some such errors of classification, if they may be so called, are not surprising. If in fact such "errors of classification" have been made in the F_2 records, then it might be expected that some of them would be brought to light in the F_3 generation. In Section E of Table III two such cases are given. In the F_2 counts these two plants are both recorded as "Very slight heart"; but the subsequent breeding shows that, on the two-factor theory, we must regard them as belonging genetically, to the class "No heart." It is worth noting that in each case the "error" has been between the classes "Very slight heart" and "No heart"; and it is admittedly the boundary between these two classes which it is in practice the most difficult to define. Furthermore, a reference to Table I, p. 369, shows that in the F_2 of 1920 from the cross Kohl Rabi by Cabbage, there is an

TABLE III.

Analysis of the grades of "Hearting" from the cross Kohl Rabi × Cabbage (1920).

Type	Genera- tion	Refer- ence*	Heart	Slight heart	Very slight heart	No heart	Total	Constitu- tion
A. Heart	F_2	6.3	141	—	—	—	141	$n_1n_1n_2n_2$
B. Slight heart	$\{F_2$	6.33	49 (62)	130 (124)	69 (62)	4	249	$N_1n_1n_2n_2$
	$\{F_2$	6.50	41 (32)	55 (64)	31 (32)	—	129	$N_1n_1n_2n_2$
	Total		88 (94)	185 (188)	100 (94)	4	377	
C. Very slight heart	$\{F_2$	6.69	1 (7)	9 (28)	56 (42)	45 (35)	111	$N_1n_1N_2n_2$
	$\{F_2$	6.42	14 (16)	68 (63)	89 (95)	80 (79)	253	$N_1n_1N_2n_2$
	Total		15 (23)	77 (91)	145 (137)	125 (114)	364	
	$\{F_2$	6.81	—	—	42	1	43	$N_1N_1n_2n_2$
	$\{F_3$	8.19	—	—	184	—	184	$N_1N_1n_2n_2$
	$\{F_3$	8.22	—	—	214	—	214	$N_1N_1n_2n_2$
	$\{F_3$	8.6	—	—	158	—	158	$N_1N_1n_2n_2$
	$\{F_2$	6.78	—	—	25 (18½)	49 (55½)	74	$N_1N_1N_2n_2$
	$\{F_2$	6.87	—	—	13 (10)	26 (29)	39	$N_1N_1N_2n_2$
	$\{F_2$	6.85	—	—	12 (10)	28 (30)	40	$N_1N_1N_2n_2$
D. No heart	$\{F_2$	6.74	—	—	5 (9)	31 (27)	36	$N_1N_1N_2n_2$
	$\{F_2$	6.15	—	6	56 (63½)	192 (190½)	254	$N_1N_1N_2n_2$
	$\{F_3$	8.16	—	—	46 (39)	110 (117)	156	$N_1N_1N_2n_2$
	$\{F_3$	7.39	—	—	17 (26)	88 (79)	105	$N_1N_1N_2n_2$
	Total		—	6	174 (173)	514 (520)	694	
	$\{F_2$	6.69	—	—	—	408	408	$N_1N_1N_2N_2$
E. Very slight heart	$\{F_2$	6.54	—	—	—	306	306	$N_1N_1N_2N_2$
	$\{F_3$	7.178	—	—	—	184	184	$N_1N_1N_2N_2$
	$\{F_2$	6.73	—	—	1	17	—	$N_1N_1N_2N_2$
	$\{F_2$	6.64	—	—	29 (25½)	73 (76½)	102	$N_1N_1N_2N_2$

* The figure to the left of the decimal point refers to the Book No., and the figures to the right of the point to the page. E.g. "7.178" means Book 7, page 178.

excess of plants in the class "Very slight heart," and a corresponding deficiency in the class "No heart," a result entirely consistent with the subsequent F_3 records. No doubt if the more or less arbitrarily chosen boundary between these two classes had been slightly shifted towards the class "No heart," more consistent figures in F_2 and F_3 would have been obtained.

It is a matter of regret that a relatively high mortality in the F_2 plants of the classes "Heart" and "Slight heart" has caused these two classes to be under-represented in the table of F_3 results; since clearly an important test of the theory here put forward is that all hearts should breed true, provided always that regard is had for the influence of environment (p. 367) and also for the effects of self-fertilisation on the vigour of the cabbage plant. The latter is an important consideration, since feeble plants rarely produce good "Hearts." Where, however,

cabbages have been bred by crossing, as they are by the seed grower, they have been found to breed true. For example, from the cross Savoy by Red Cabbage, the progeny both in F_1 and F_2 were all plants with good hearts.

To sum up, therefore, it may be said that the F_2 results pointed to a two-factor theory, and this has been confirmed by the records of the Back crosses and of the F_3 and F_4 cultures. It is more than likely that there are other minor factors as well, which modify the degree of hearting and which an exact method of classification and strictly uniform cultural conditions might bring to light. But that there are two principal factors involved seems a reasonable conclusion to be drawn from the breeding analysis. No account has as yet been taken of the factors which determine the shape of the heart.

The Relation of the N factors to other factors.

(i) *The Factor D.*

The difference between the ordinary green *oleracea* on the one hand, and the purple coloration of the "Red" Cabbage and the "Blue" Vienna Kohl Rabi on the other, is usually determined by a single Mendelian factor which Kristofferson has designated *D*. The dominance is by no means "perfect," the heterozygote usually being paler in colour than the pure type. Segregation is generally quite sharp; only now and again does a plant turn up which gives difficulty in classification, owing to the fact that in hot dry weather the leaf stalks and axils of most *oleracea* plants turn a slight red tinge.

The *D* factor seems to be quite independent of the *N* factors, as the following table shows.

TABLE IV.

Showing free assortment between colour and hearting.

F_2 . Green Cabbage \times Purple Kohl Rabi.

$ddn_1n_1n_2n_2 \times DDN_1N_1N_2N_2$.

(Bk. 5, p. 18, 1926.)

	Heart	Slight heart	Very slight heart	No heart	Total
Green	15 (10)	43 (41)	54 (62)	47 (52)	159 (165)
Purple	28 (31)	124 (124)	217 (186)	132 (155)	501 (496)
Total	43 (41½)	167 (165½)	271 (248)	179 (207)	661

It is true that there is a slight excess of the class "Green : Heart" which might indicate slight linkage between *D* and N_1 or N_2 , were it

not for the fact that the corresponding class "Purple : No heart" shows a deficiency.

(ii) *The factors P and E.*

Although there are striking differences of type and form in the leaves of the *oleracea* races, the genetics of these characters has proved to be a difficult and rather unsatisfactory matter. For not only are the characters studied elusive of exact definition, but also the type of foliage thrown up changes with the age of the plant.

In the cabbage parents used for these experiments, the leaves in the normal full-grown plant are broad, rounded, entire, and sessile (Plate XXXI, fig. 2); the Kohl Rabi has leaves which are narrow, pointed, petiolate and roughly lyrate in form (Plate XXXI, fig. 1). But in both cases, the young plants have leaves which are petiolate and the flowering shoot has leaves which are sessile and entire, the transitions in neither case being sharp. The basal leaves, even in the full grown cabbage plant, will often have a short petiole persisting, and in the Kohl Rabi, the youngest leaves of the rosette will often be found to have a very reduced petiole or even none. Thus even in the pure parent plants, in which, at first glance, the difference of type of the normal foliage is so very striking, it is difficult to make an absolute distinction. And it is not surprising, therefore, that the intermediate plants in an F_2 culture give types of foliage which it is very difficult to classify exactly. The principal difficulty arises from the fact that in many cases all types of foliage in every stage of transition appear on one and the same plant, sometimes simultaneously, but more often the predominating leaf form changes as the season advances. It was not till a large number of F_3 cultures had been raised that it became at all possible to get some idea of the factors at work and to draw up what may perhaps be called the rules of recording. These "rules" are as follows. The counting must be done only on plants which have reached the normal mature resting stage and which have not yet started to show signs of spring growth. In practice this usually means from November till the middle of January. The leaves by which the foliage type is to be judged must be those which are half-way between the basal leaves and the growing point. If this scheme of recording is adhered to, it is found that the foliage of the F_2 plants from the cross Kohl Rabi by Cabbage fall in three groups as to type, namely, (1) Petiolate Lyrate, (2) Petiolate Entire and (3) Sessile Entire (Plate XXXI, figs. 1-3), in roughly the ratio 1 : 2 : 1. At first sight this would seem to indicate a single factor difference; but there are two considerations which point to

there being in reality two closely-linked factors at work. For if there were only one factor, then the leaf type Petiolate Entire should always be heterozygous; but in the Brussels Sprout we have leaves which are Petiolate Entire and the plants nevertheless breed true. The other consideration is that on three occasions leaves have been recorded in F_2 cultures as "Sessile Lyrate¹." The appearance of this fourth class is inexplicable on a single-factor theory; but it would be expected to occur if there were two factors involved. It therefore seems more consistent to suppose that there is one factor P which causes the foliage type to be petiolate as against sessile, and another factor E which causes the leaves to be entire as opposed to lyrate. In the case of P , the dominance is pronounced; but with E , the heterozygote is inclined to be intermediate, in some crosses much more so than in others (cf. Plate XXIX, figs. 3-6). These two factors P and E are very closely linked together, and the cross-over classes are so rare as to be negligible in the ordinary F_2 counts.

It is interesting to notice that in the wild *B. oleracea*, a very common type of foliage in the one-year-old plants is Petiolate Entire, which would point to a factorial constitution $PPEE$. The foliage of the Cabbage ($ppEE$) and of the Kales and Kohl Rabi ($Ppee$) may therefore be supposed to have originated from such a wild type by the loss of the factors P and E respectively; while the Brussels Sprout maintains the original combination $PPEE$. The rare type of foliage "Sessile : Lyrate" is presumably $ppee$, having originated by means of a cross-over in the hybrid Pe, pE .

It is not claimed that even in the cross Kohl Rabi by Cabbage these two factors account for all the transition forms which appear in F_2 (Plate XXXI, figs. 4-8). There are doubtless minor factors which careful breeding would bring to light. But the main types of foliage found in this cross can be explained with the help of the two factors P and E , which in this case act to all intents and purposes as a single factor. It is not intended to enter into a full discussion of all the foliage types in *oleracea* at this stage: it is sufficient for the moment to have defined the factors P and E , in order to determine their relation to the N factors.

This relationship is illustrated in the following table, showing the distribution of types in the F_2 from the cross Savoy Cabbage by Kohl Rabi.

It is clear from this table that there is not free assortment between the factors P and E on the one hand and the N factors on the other. All the "Hearts" have sessile entire foliage and out of the 83 "No

¹ But so far I have not obtained any progeny from this type.

TABLE V.

Showing the linkage between N_1 and P and E . *F_2 . Savoy Cabbage \times Kohl Rabi.* *$n_1n_1n_2n_2ppEE \times N_1N_1N_2N_2PPee$.*

(Bk. 8, p. 68, 1924.)

	Heart	Slight heart	Very slight heart	No heart	Total
Petiolate Lyrate	— (·1)	2 (2)	17 (15)	34 (34)	53 (52)
Petiolate Entire	— (2)	13 (26)	49 (48)	47 (28)	109 (104)
Sessile Entire	8 (11)	21 (24)	15 (15)	2 (2)	46 (52)
Total	8 (13)	36 (52)	81 (78)	83 (65)	208

hearts" only two have sessile entire leaves. From what has gone before, it must be presumed that there is no linkage between N_1 and N_2 , nor has anything so far come to light to distinguish the one factor from the other in any way whatever. Let us therefore give to the factor which is linked to P and E the designation N_1 . If the gametic series between P and N_1 is given by $xPN_1 : 1pN_1 : 1Pn_1 : xpn_1$, then it is easy to work out the generalised distribution of types in the F_2 and deduce the value of x by comparison with the figures found by experiment. In this way it can be calculated that to the nearest whole number, $x = 10$ gives the best fit. The expectation on this hypothesis is shown by the figures in italics in Table V. The discrepancies which call for comment are firstly, that the class "Slight heart : Sessile, entire" is in excess of the class "Slight heart : Petiolate, entire," where nearly equality is expected on the theory; and secondly, that the class "No heart : Petiolate, entire" is in excess of the class "No heart : Petiolate, lyrate," where the theory demands the opposite of this. All that can be said at present is that in this culture the totals of the heart classes themselves show some divergence from expectation; and that in any case the numbers are small, and more data will have to be collected to find out if these discrepancies are persistent from year to year. At all events, linkage between the factors P and N_1 has been clearly shown, though an exact evaluation of the cross-over percentage is not yet possible.

(iii) *The Factor W.*

Apart from the leaf type, there is a very striking difference in the breadth of the Cabbage leaf as compared with that of the Kohl Rabi (Plate XXXI, figs. 1 and 2). The F_1 leaf, though intermediate, is distinctly towards the broad size (Plate XXXI, fig. 3). In the F_2 the segregation is not sharp; but when the plants with leaves about as narrow as the parent Kohl Rabi are

picked out they are found to form about a quarter of the total. The F_3 analysis confirms the view that there is one principal factor involved in this character difference, though here again there are probably minor modifying factors which patient breeding would identify. There were five F_2 plants recorded as narrow which were grown on to F_3 and each bred true to narrowness (783 plants), none giving any offspring with intermediate or broad leaves. Of the twelve "Broad" F_2 plants grown on, seven gave both types of leaf (457 broad, 180 narrow) and five bred true to broadness (441 plants). Hence we may conclude that there is certainly one principal factor, W , involved in this character difference.

The relation of this factor W to the N factors is shown in the following table, which gives the distribution of the types in question in the F_2 from the cross Cabbage ($n_1n_1n_2n_2WW$) by Kohl Rabi ($N_1N_1N_2N_2ww$).

TABLE VI.

Showing linkage between Broad Leaves and Heart. The expectation is calculated on the hypothesis that the gametic series is $3Wn : 1WN : 1wn : 3wN$.

F_2 . Savoy Cabbage \times Kohl Rabi.

$n_1n_1n_2n_2WW \times N_1N_1N_2N_2ww$.

(Bk. 8, p. 6, 1924.)

	Heart	Slight heart	Very slight heart	No heart	Total
Broad Leaves	8 (12)	35 (46)	74 (60)	47 (37)	164 (156)
Narrow Leaves	— (1)	1 (6)	7 (18)	36 (28)	44 (52)
Total	8 (13)	36 (52)	81 (78)	83 (65)	208

Here there is clearly association between broad leaves and heart on the one hand, and narrow leaves and no heart on the other. It is not possible from this table to say whether W is linked to N_1 or to N_2 . But whichever it may be, a gametic series $1WN : 3Wn : 3wN : 1wn$ gives a tolerably close fit. The most noticeable discrepancy is that the plants with narrow leaves show a deficiency at the heart end of the scale, while there is no corresponding deficiency in the broad leaved class at the no-heart end of the scale. Here again, further breeding will be necessary to find out if this asymmetrical distribution is persistent.

The question as to whether W is linked to N_1 or to N_2 can be settled by a consideration of the distribution of broad and narrow leaves between the classes petiolate and sessile. For if W turns out to be linked to P , then, since P is linked to N_1 (p. 275), it follows that W will be also linked to N_1 . On the other hand, if W shows no linkage with P ,

then the distribution of types shown in Table VI is presumably the result of linkage between W and N_2 .

A consideration of the following table, showing the record of leaf types in the F_2 from the cross Cabbage by Kohl Rabi, settles the point.

TABLE VII.

Showing the linkage between Petiolate and Narrowness on the one hand and Sessile and Broadness in leaves on the other. The expectation is calculated on the hypothesis that the gametic series is $1WP : 2Wp : 2wP : 1wp$.

F_2 . Savoy Cabbage \times Kohl Rabi.

$WWppEE \times wwPPee$.

(Bk. 8, p. 6, 1924.)

	Petiolate Lyrate	Petiolate Entire	Sessile Entire	Total
Broad Leaves	34 (31)	89 (86)	50 (50)	173 (167)
Narrow Leaves	23 (25)	25 (25)	2 (6)	50 (56)
Total	57 (56)	114 (111)	52 (56)	223

It is clear at once that the broad leaves are nearly always sessile and the narrow leaves predominantly petiolate. Hence we may conclude that W is linked to P ; and, as will be seen from Table VII, a gametic series $1WP : 2Wp : 2wP : 1wp$ gives a good fit which calls for no special comment. Thus, since W is linked to P , we may conclude that W is also linked to N_1 and is independent of N_2 .

(iv) *The Factor T.*

As regards habit of growth, the *oleracea* species is found in tall and sessile varieties. The Cabbage is usually dwarf or sessile, but there are a few tall or upright races, in which the heart is borne at the end of a stalk $1\frac{1}{2}$ to 2 feet in height. Brussels Sprouts, as usually grown, are always tall, as are also Thousand-headed Kale, and most other Kales; but the Curly or Scotch Kale is often grown in a sessile or dwarf form. Kohl Rabi is always sessile, but it is possible that the Marrow Stem Kale will turn out to be the tall form of Kohl Rabi. Tall is dominant to dwarf, and the character usually segregates fairly clearly. That this broad distinction of habit is a matter of one factor can be seen from the following table, which shows the equality of types obtained by crossing the tall F_1 hybrids with the dwarf parent form.

TABLE VIII.

Back cross. F_1 (*Tall* \times *Dwarf*) \times *Dwarf* (1924).

	<i>Tt</i> \times <i>tt</i> .	Reference	Tall	Dwarf	Total
F_1 (Brussels Sprouts \times Cabbage) \times Cabbage		Bk. 8, p. 38	198	204	402
F_1 (Cabbage \times Curly Kale) \times Cabbage		Bk. 7, p. 181	46	53	99
Total	—		244 (250½)	257 (250½)	501

In order to ascertain whether *T* is linked to either of the *N* factors, it is only necessary to set out in detail the second Back cross given in Table VIII. This is shown by Table IX, which makes it quite clear that there is association between heart and dwarf habit on the one hand, and no-heart and tallness on the other.

TABLE IX.

Showing the linkage between *T* and *N*.Back cross. F_1 (Cabbage \times Curly Kale) \times Cabbage. $N_1n_1N_2n_2Tt \times n_1n_1n_2n_2tt$.

(Bk. 7, p. 181.)

	Heart	Slight heart	Very slight heart	No heart	Total
Tall	5	29	12	—	46 (49½)
Dwarf	20	24	9	—	53 (49½)
Total	25 (25)	53 (49)	21 (25)	—	99

From this table it will be seen that there are $5 + 9 = 14$ cross-over gametes, and $12 + 20 = 32$ non-cross-over gametes, which would give a cross-over value of about 30 per cent.

As in the case of the factor *W*, it now remains to ascertain whether *T* is linked to N_1 or to N_2 . This can be settled by finding out the relation of *T* to the factor *P*: the most convenient material for this is the Back cross given in Table IX, rearranged so as to give the distribution of sessile and petiolate foliage between tall and dwarf plants. This is set out in Table X.

TABLE X.

Showing the absence of linkage between *T* and *P*.Back cross. F_1 (Cabbage \times Curly Kale) \times Cabbage. $TtPpEe \times ttppEE$.

(Bk. 7, p. 181.)

	Leaves Sessile : Entire	Leaves Petiolate : Entire	Total
Dwarf	34	23	57
Tall	25	21	46
Total	59	44	103

If T is not linked to P , we should expect an equal number of plants in all classes from this Back cross; and, as Table X shows, this is roughly what we have obtained by experiment. It is true that there is an excess of the class Dwarf: Sessile; but if this were taken to be the result of linkage, we should expect a corresponding excess in the class Tall: Petiolate; whereas, in fact, there is a deficiency in this class. The numbers are small, but, as far as they go, they point to free assortment between P and T . Hence if T is not linked to P , it is by hypothesis also not linked to N_1 . The association between heart and dwarf habit must therefore be due to linkage between T and N_2 .

(v) *The Factor A.**

There is a queer monstrosity of the leaf in which outgrowths occur from the veins (particularly from the midrib) at right angles to the surface of the leaf (Plate XXX, fig. 9). This anomalous growth is usually of a leafy nature, but in gross cases the structure becomes stalky and even bears flowers and sets seed. The earliest mention of this form is by Gerarde¹, who says it "hath many great and large leaves whereupon do grow heere and there other small jagged leaves, as it were made of ragged shreds and jagges set upon the smooth leafe, which giveth shewe of a plume or fanne of feathers." It is also recorded by Bauhinus, who says it was known to the Greeks, who called it *Brassica asparagodes*².

In crossing, the "Asparagodes" character (if we may so call it) behaves as a simple dominant, the heterozygote showing rather less of the malformation than the homozygote. There is one record of an F_2 from the cross "Asparagodes" Kale by Cabbage, which is set out in

TABLE XI.

Showing free assortment in F_2 between A and N .

F_2 . *Asparagodes Kales* \times *Cabbage*.

$AA N_1 N_1 N_2 N_2 \times aa n_1 n_1 n_2 n_2$.

(Bk. 6, p. 115, 1922.)

	Heart	Slight heart	Very slight heart	No heart	Total
Asparagodes Foliage	6	18	15	17	56 (54)
Normal Foliage	1	5	8	2	16 (18)
Total	7 ($4\frac{1}{2}$)	23 (18)	23 (27)	19 ($22\frac{1}{2}$)	72

¹ *The Herball of John Gerarde*, London, 1597, p. 243.

² *Historia Plantarum*, Geneva, 1651, p. 833. It should perhaps be added that no support for this view is forthcoming from Aristotle, who does not include any such form in his account of the *Brassicae*.

Table XI. From this, though the numbers are small, it can be seen that there is no linkage between the Asparagodes factor, *A*, and either of the *N* factors.

(vi) *The Curly foliage of Scotch Kale.*

As we have seen from the experiments of Malinowski and Kristoffer-son, the inheritance of the curly foliage of Scotch Kale is no simple matter, a conclusion which my own experiments have amply confirmed. In classifying the plants, I have attempted to define three intermediate grades, making, with the parent types, five classes in all (Plate XXXI, figs. 9-14). The F_1 is uniform and of about Grade II in all cases of the cross between full curly and smooth (Plate XXXI, fig. 11). The distribution into these five classes of the F_2 plants for all cultures from 1920-4 is as follows:

TABLE XII.

F_2 . *Curly* \times *Smooth Leaves* (total 1920-4).

Total	Smooth	Intermediate			Curly
		Grade I	Grade II	Grade III	
4064	85	1268	2614	101	1

Evidently there are many factors involved: it is not proposed at present to discuss the genetics of this character in general. Experiments are in progress which, it is hoped, will enable the factors involved to be defined. But the relation of this character to the heart of the cabbage has, as we have seen, been treated by Malinowski and it is, perhaps, worth while to see how far his results agree with the Cambridge experi-

TABLE XIII.

Showing the association of Heart and Smoothness as against No heart and the higher grades of Curliness.

F_2 . *Cabbage* \times *Curly Kale*.

$n_1 n_1 n_2 n_2 k_1 k_1 k_2 k_2 \dots \times N_1 N_1 N_2 N_2 K_1 K_1 K_2 K_2 \dots$

(Bk. 6, p. 13, 1921.)

	Smooth	Intermediate		Curly	Total
		Grades I and II	Grade III		
Heart	3	6	1	—	10 (4)
Slight heart	2	2	10	—	14 (17)
Very slight heart	3	9	17	—	29 (26)
No heart	—	5	12	—	17 (22)
Total	8	22	40	—	70

ments. Unfortunately, although I have made many crosses involving the curly character, there is only one case of an F_2 from the cross Cabbage by Curly Kale, and the culture is a small one. But it is significant. In Table XIII this F_2 is set out so as to show the relationship of the curly foliage to the hearting character.

Clearly there is association between Hearting and Smoothness on the one hand, and No heart and Curliness on the other; though in this experiment the association is neither rigid nor symmetrical as Malinowski found in his material. In order to make the comparison clear, I have set out on a similar correlation diagram the figures given on p. 3 of Malinowski's paper. This is shown by Table XIV.

TABLE XIV.

Showing the strict and symmetrical association between Hearting and Smoothness according to Malinowski (cf. Table XIII).

F_2 . Curly Kale \times Cabbage.

		Grade of Curliness						
		1	2	3	4	5	6	7
Grade of Hearding	1	9	—	—	—	—	—	—
	2	—	57	—	—	—	—	—
	3	—	—	113	—	—	—	—
	4	—	—	—	220	—	—	—
	5	—	—	—	—	110	—	—
	6	—	—	—	—	—	64	—
	7	—	—	—	—	—	—	11

Such a distribution would indicate an equal number of strictly polymeric factors for each character, and intense coupling between each pair of factors. At all events, the numbers which I have so far obtained from this cross, small though they be, clearly show that Malinowski's hypothesis is not applicable to the Cambridge material. My figures do, however, point distinctly to some sort of association between smoothness and heart as against curliness and absence of heart. Kristofferson's figures, as has already been mentioned, have not been recorded so as to be of any use in a discussion on linkage. However, my own small F_2 can be supplemented by the relevant Back crosses, which are set out in Tables XV and XVI.

In the case of the Back cross $F_1 \times$ Curly Kale (Table XV), the linkage does not appear very strikingly, the most significant thing being the relatively small number of plants in the class "Very slight heart: Smooth." On the other hand, in the converse Back cross $F_1 \times$ Cabbage (Table XVI), the skew distribution is very marked. Of the 65 plants in the class "No heart," no less than 60 are recorded fully as curly as the

TABLE XV.

Showing linkage between N and K in the two Back crosses. F_1 (Cabbage \times Curly Kale) \times Curly Kale. $N_1 n_1 \tilde{N}_2 n_2 K_1 k_1 \dots \times N_1 N_1 N_2 N_2 K_1 K_1 \dots$

(Bk. 7, p. 181, 1924.)

	Heart	Slight heart	Very slight heart	No heart	Total
Smooth	11	24	4	—	39
Intermediate:					
Grade I	14	29	17	—	60
Grade II	—	—	—	—	—
Grade III	—	—	—	—	—
Curly	—	—	—	—	—
Total	25 (25)	53 (49)	21 (25)	—	99

TABLE XVI.

Showing linkage between N and K in the two Back crosses. F_1 (Cabbage \times Curly Kale) \times Cabbage. $N_1 n_1 N_2 n_2 K_1 k_1 \dots \times n_1 n_1 n_2 n_2 k_1 k_2 \dots$

(Bk. 8, p. 44, 1924.)

	Heart	Slight heart	Very slight heart	No heart	Total
Smooth	—	—	—	—	—
Intermediate:					
Grade I	—	—	1	—	1
Grade II	—	—	17	7	24
Grade III	—	—	44	109	153
Curly	—	—	5	60	65
Total	—	—	67 (61)	176 (182)	243

parent type (and this notwithstanding the fact that in the straight F_2 only one in 4000 of this class turned up) and the remaining five all fall into the highest grade of intermediate type of curliness.

Unfortunately the factorial analysis of curliness has turned out to be unexpectedly complex and it will be more profitable to defer a discussion of the genetics of this character till further data have been collected. For the present discussion which, after all, centres round cabbages, it will suffice to suppose that there are several multiple factors ($K_1, K_2, K_3 \dots$) which determine the curly character of the leaves of Scotch Kale. But until these factors have been exactly defined and identified by breeding experiments, it is impossible to give a complete description of the association between hearting and smoothness, in the cross Cabbage by Curly Kale, in terms of factors and cross-over per-

centages. This much, however, we can say, that at least one of the curly factors is linked to one of the hearting factors, though it is more than likely that the linkage will turn out to be more complex. Clearly what we have to determine is whether the chromosome make up is of the type

$$\begin{array}{c|c} \begin{array}{c} -N_1 \\ -K_1 \end{array} & \begin{array}{c} -N_2 \\ -K_2 \end{array} \end{array} \text{ or } \begin{array}{c|c} \begin{array}{c} -N_1 \\ -K_1 \end{array} & \begin{array}{c} -N_2 \\ -K_2 \end{array} \end{array} \text{ or } \begin{array}{c|c} \begin{array}{c} -N_1 \\ -K_1 \end{array} & \begin{array}{c} -N_2 \\ -K_2 \end{array} \end{array}$$

(leaving out of consideration for the time being other possible K factors). The figures which we have so far considered do not throw light on this problem. But, as before, we must have recourse to some other factor, whose relationship to the N factors has already been independently established. Such a factor is T , which determines the difference between tall and sessile habit; and in the Back cross F_1 (Cabbage \times Curly Kale) \times Cabbage we have the relevant figures. These are set out in Table XVII.

TABLE XVII.

Showing slight linkage between T and K .

Back cross. F_1 (Cabbage \times Curly Kale) \times Cabbage.

$TtK_1k_1K_2k_2 \dots \times ttK_1k_1K_2k_2 \dots$

(Bk. 7, p. 181, 1924.)

	Smooth	Intermediate			Curly	Total
		Grade I	Grade II	Grade III		
Tall	13	33	—	—	—	46 ($49\frac{1}{2}$)
Dwarf (sessile)	22	31	—	—	—	53 ($49\frac{1}{2}$)
Total	35	64	—	—	—	99

The numbers are small, but they would seem to point to a slight linkage between T and one of the K factors (which we may designate as K_2). If there is no linkage between T and any of the other K factors, it can be seen from the figures in Table XVII that the cross-over value between T and K_2 is $\frac{13 + 31}{99} = 44$ per cent. Clearly the experimental

numbers so far obtained are too small to furnish convincing evidence of this linkage. But if further data should confirm this linkage, then it would follow, since T is linked to N_2 , that K_2 is also linked to N_2 . Even so, that does not exclude the possibility of another K factor (say K_1) being linked to N_1 . In fact there is evidence of this being the case from the crosses Kohl Rabi \times Cabbage and Kohl Rabi \times Curly Kale, on which it is hoped to report in a subsequent communication.

SUMMARY.

1. There are two independent factors, N_1 and N_2 , which are normally present in the Kale type of *B. oleracea*; when both these factors are absent, the common Cabbage heart develops under normal cultural conditions. Intermediate degrees of "hearting" are produced by one or two "doses" of N .

2. The Heart character shows "linkage" with a number of other characters. Clearly it is of interest to determine, in each case, whether linkage is with the factor N_1 or with N_2 .

3. There is one linkage group comprising the factors N_1 , P for petiolate leaves as against sessile, E for entire leaves as against lyrate, and W which determines broad as distinct from narrow leaves. There is some evidence that there is also in this group a factor K_1 , one of the multiple factors for the Curly foliage of Scotch Kale.

4. There is a second linkage group containing the factors N_2 , T for tall habit as against sessile habit, and probably the factor K_2 of the curly series.

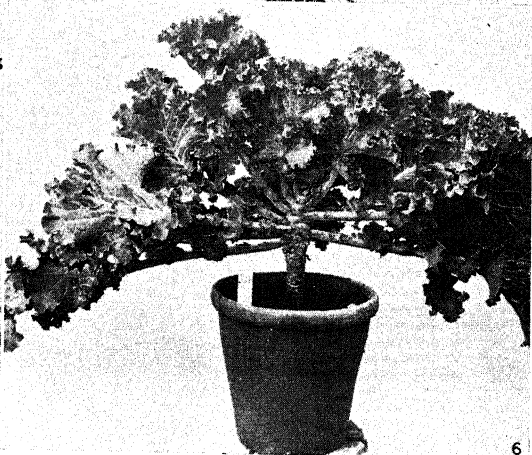
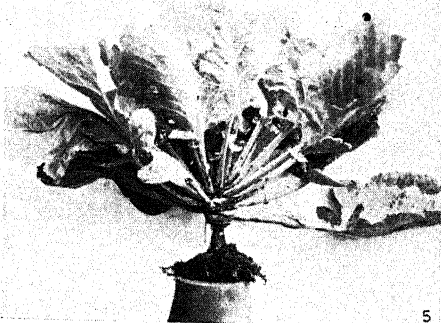
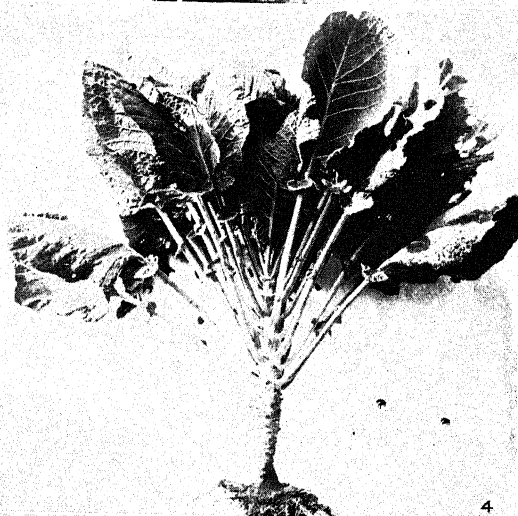
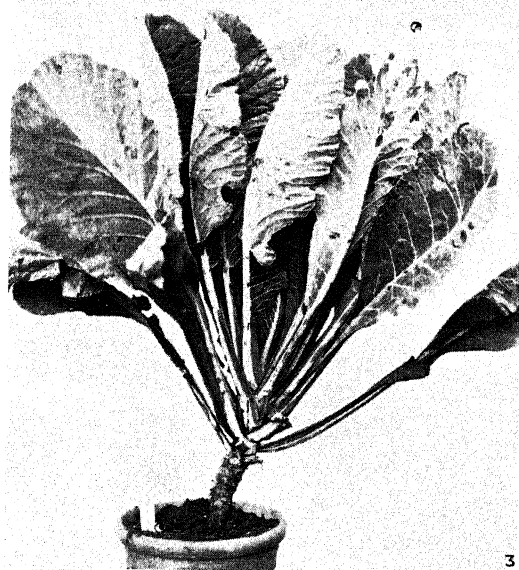
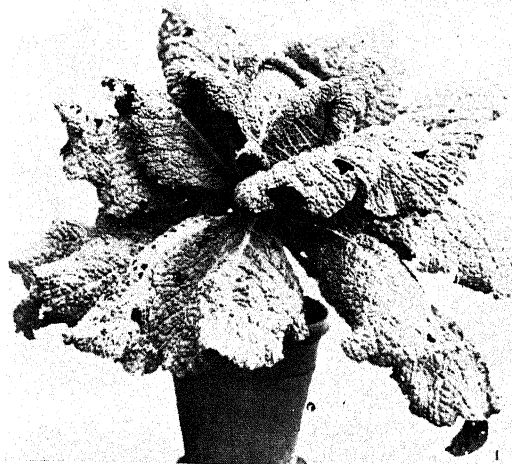
5. There are two so far unattached factors, one of which, D , differentiates between the purple and green types of *oleracea*; the other, A , determines the "Asparagodes" malformation of the foliage.

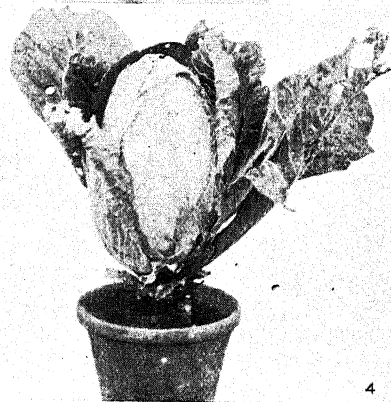
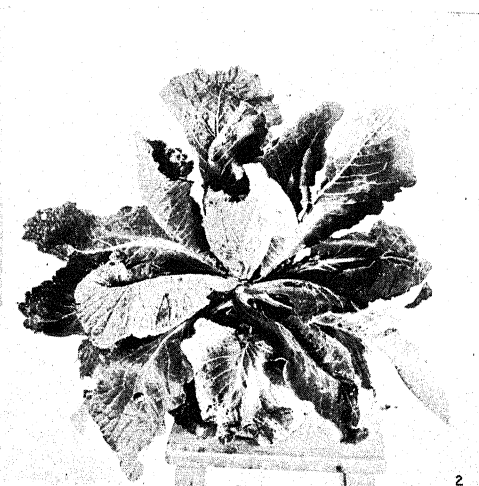
6. Thus the ten factors so far considered account for four of the nine chromosomes of *B. oleracea*.

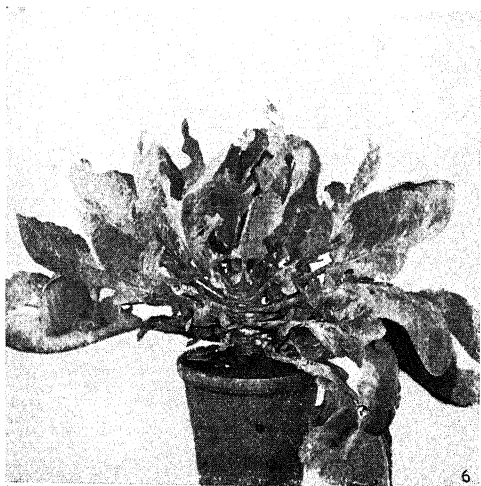
I am deeply indebted to Prof. Sir Rowland Biffen, who has made these experiments possible by placing at my disposal very generous accommodation at the Cambridge Plant Breeding Institute.

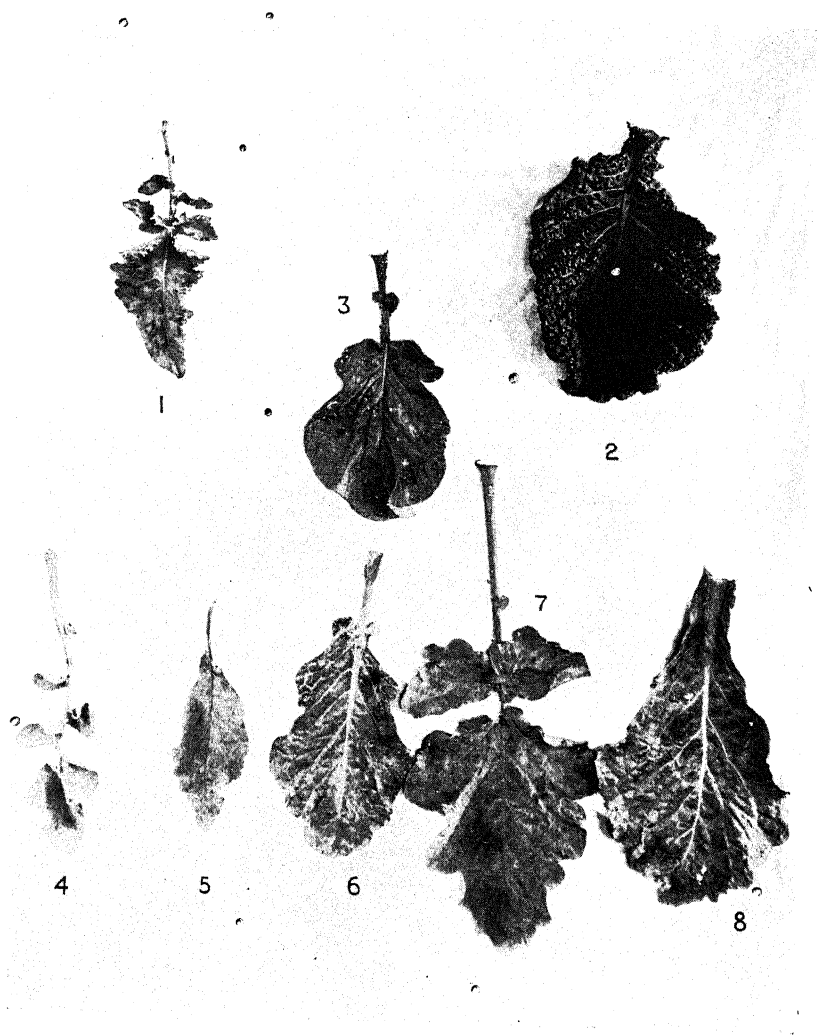
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13



14

EXPLANATION OF PLATES XXIX—XXXI.

PLATE XXIX.

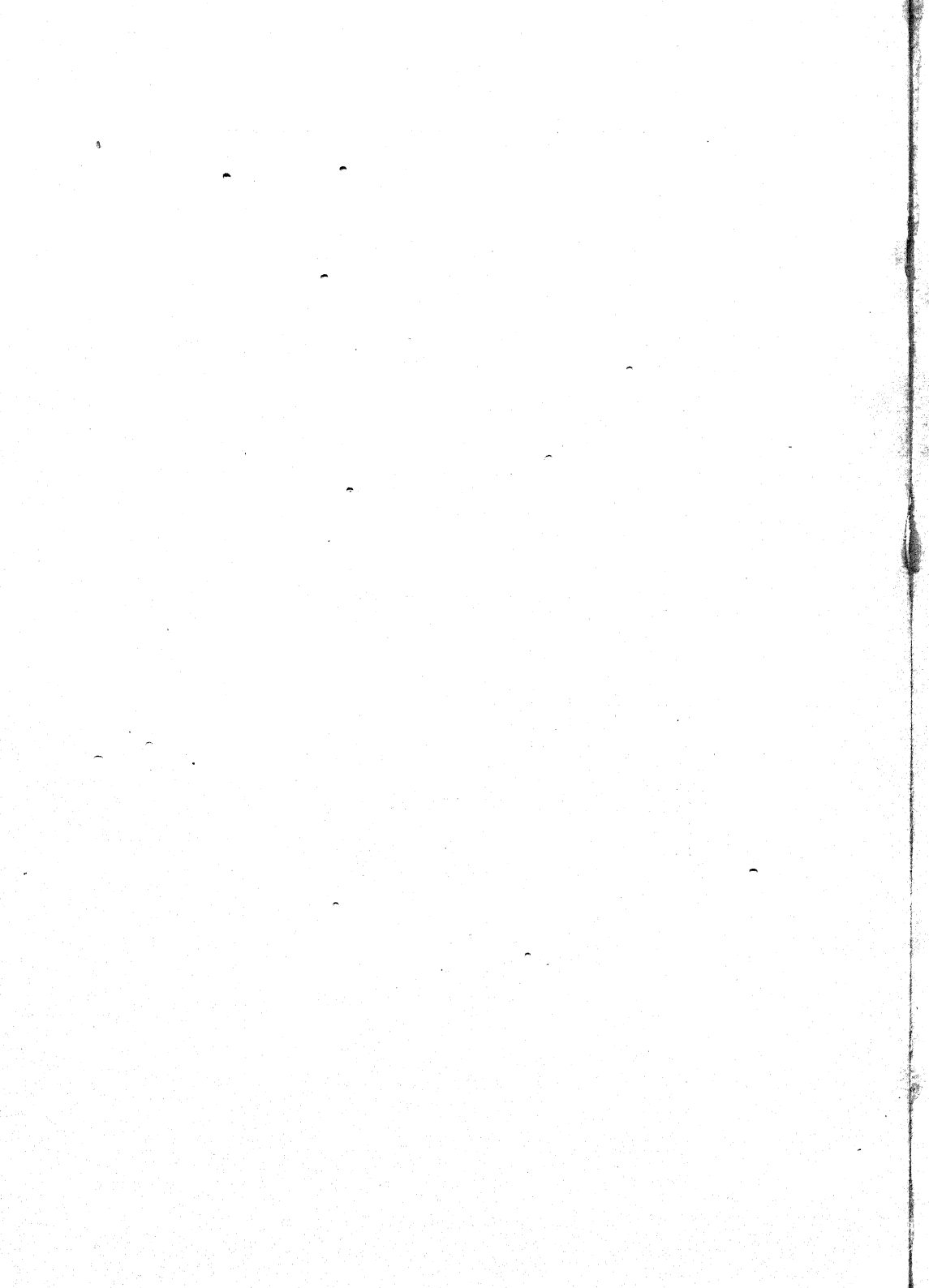
- Fig. 1. Savoy Cabbage.
- Fig. 2. Thousand-headed Kale. (A non-hearting type of *oleracea*.)
- Fig. 3. F_1 Cabbage \times Thousand-headed Kale.
- Fig. 4. F_1 Thousand-headed Kale \times Savoy Cabbage.
- Fig. 5. F_1 Kohl Rabi \times Cabbage.
- Fig. 6. F_1 Curly Kale \times Savoy Cabbage.

PLATE XXX.

- Fig. 1. F_2 Savoy \times Kohl Rabi, type "Heart."
- Fig. 2. F_2 Savoy \times Kohl Rabi, type "Slight heart."
- Fig. 3. F_2 Savoy \times Kohl Rabi, type "Very slight heart."
- Fig. 4. F_2 Kohl Rabi \times Cabbage, type "Heart."
- Fig. 5. F_2 Kohl Rabi \times Cabbage, type "Slight heart."
- Fig. 6. F_2 Kohl Rabi \times Cabbage, type "Very slight heart."
- Fig. 7. F_2 Savoy \times Kohl Rabi, type "No heart."
- Fig. 8. F_2 Kohl Rabi \times Cabbage, type "No heart."
- Fig. 9. *Brassica Asparagodes*.

PLATE XXXI. Leaf types.

- Fig. 1. Petiolate : lyrate : narrow (Kohl Rabi).
- Fig. 2. Sessile : entire : broad (Savoy Cabbage).
- Fig. 3. Petiolate : entire : broad (F_1 type).
- Fig. 4. Petiolate : lyrate : narrow (from the F_2).
- Fig. 5. Petiolate : entire : narrow (from the F_2).
- Fig. 6. Petiolate : entire : broad (from the F_2).
- Fig. 7. Petiolate : lyrate : broad (from the F_2).
- Fig. 8. Sessile : entire : broad (from the F_2).
- Fig. 9. Smooth (Kohl Rabi).
- Fig. 10. Curly (Scotch Kale).
- Fig. 11. Intermediate Grade II (F_1 type).
- Fig. 12. Intermediate Grade I (from the F_2).
- Fig. 13. Intermediate Grade II (from the F_2).
- Fig. 14. Intermediate Grade III (from the F_2).



HYPERGLYCAEMIA AS A MENDELIAN RECESSIVE CHARACTER IN MICE.

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IN the course of an investigation into the factors controlling the sugar content of the blood in mammals, commenced some years ago, we found that if the effects of fright and shock were guarded against by using only animals accustomed to being handled, the percentage after a twelve to twenty-four hours fast rarely varied more than about 5 mg. from a mean average of 85 mg. per 100 c.c. when self-coloured or piebald animals were considered, but that albinos and animals which were uniformly black frequently gave results differing by 25 mg. or more from this average, the former showing high and the latter low figures. These exceptional levels for the fasting blood sugar were so regularly encountered that it was evident they were not merely the result of accident, and it seemed probable there was some hitherto unrecognised relation between the factors determining colour and those controlling the sugar content of the blood. As our records showed that albinos did not invariably possess high fasting blood sugars, and that in black animals the percentage of sugar in the fasting blood was not always below the average, it was clear that the colour factors and the sugar factors were not identical, although the frequent association of abnormalities of colour with pronounced deviations from the mean average of sugar in the blood suggested some connection. In order to discover, if possible, the law underlying this apparent relation we commenced a series of breeding experiments, using mice for the purpose. These animals were selected because they multiply rapidly, occupy little space, and a large volume of work has already been published concerning colour transmission in them. They present the disadvantage, however, that, owing to their small size, it is necessary to kill the animal to obtain sufficient material for a reliable determination of the percentage of sugar in the blood, so that if death occurs naturally or by accident before a breeding experiment is completed a series of observations may be nullified. This happened in several of our series, but a sufficient

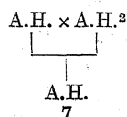
number of completed observations have been made to warrant conclusions being drawn regarding the relation between albinism and hyperglycaemia, and for the present we shall confine ourselves to that aspect of the question.

As will presently appear, the experiments showed no genetical connection between albinism and high sugar percentage, and the association first observed must consequently be regarded as fortuitous.

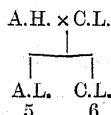
In all cases the blood required for the sugar estimations was obtained by decapitating the animal and the determinations were carried out by a modification of the Folin and Wu process, employing 0.2 c.c. of blood, which we have described elsewhere¹.

First Generation.

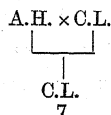
Family A. A mating between two albino mice, both possessing high fasting blood sugars of 116 mg. and 120 mg. respectively, yielded a family of seven, all albinos with high blood sugars ranging from 114 to 124 mg. per 100 c.c.



Family B. By mating ♂ albino having a high blood sugar, 120 mg., with ♀ black and white piebald having a low blood sugar, 80 mg., we obtained a second family consisting of five albinos showing low blood sugars, 76 to 84 mg., and six black and white piebalds also showing low blood sugars, 76 to 80 mg.



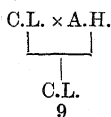
Family C. A third family consisting of seven chocolate coloured animals, all with low blood sugars ranging from 78 to 86 mg., resulted from the crossing of ♂ albino having a high blood sugar, 118 mg., with ♀ chocolate having a low blood sugar, 76 mg.



¹ *New Views on Diabetes*, Oxford Med. Publications, p. 5, 1923.

² A., albino; C., coloured; H., high; L., low.

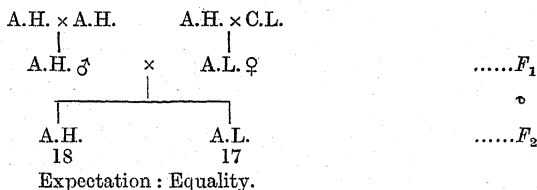
Family D. By crossing ♀ albino having a high blood sugar, 118 mg., with ♂ piebald chocolate and white having a low blood sugar, 84 mg., we obtained a fourth family of nine, all piebalds, showing low blood sugars ranging from 76 to 84 mg.



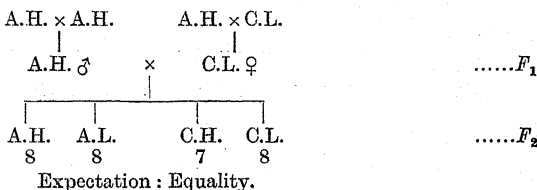
These initial results indicated that a high blood sugar behaves as a recessive to a normal blood sugar in the same way as albinism behaves to colour. In the families *A*, *C* and *D* both parents were obviously homozygous for colour and for sugar, but in family *B* the coloured partner was apparently heterozygous for colour, although homozygous for sugar, thus explaining the appearance in their progeny of approximately equal numbers of albinos and coloured animals, all with low blood sugars.

Second Generation.

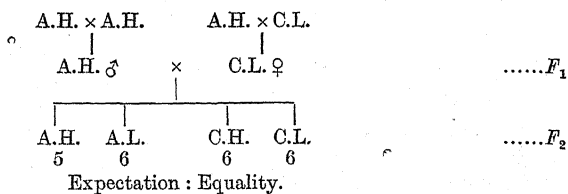
Family E. On mating ♂ albino having a high blood sugar, 120 mg., from family *A*, with ♀ albino having a low blood sugar, 80 mg., from family *B*, we obtained 35 individuals, 18 of which were albinos with high blood sugars, 114 to 124 mg., and 17 were albinos with low blood sugars, 76 to 84 mg.



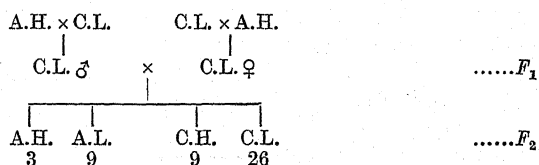
Family F. A cross between one of the ♂ high blood sugar albinos, 116 mg., of family *A* with a ♀ low blood sugar piebald, 80 mg., from family *B*, gave 23 mice consisting of 8 albinos with high blood sugars, 116 to 122 mg., 8 albinos with low blood sugars, 76 to 85 mg., 7 piebalds with high blood sugars, 116 to 120 mg., and 8 piebalds with low blood sugars, 80 to 84 mg.



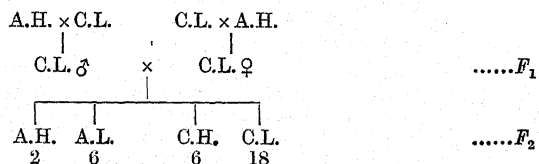
Family G. A high blood sugar albino, 114 mg., from family *A* on being mated with a low blood sugar black and white piebald, 78 mg., from family *B* gave a family of 23, 5 albinos having high blood sugars, 114 to 120 mg., 6 albinos having low blood sugars, 76 to 84 mg., 6 piebalds having high blood sugars, 116 to 122 mg., and 6 piebalds having low blood sugars, 80 to 84 mg.



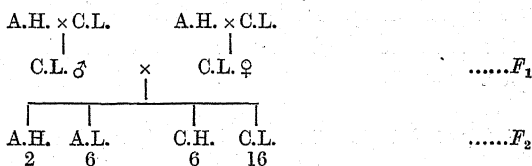
Family H. The crossing of a chocolate coloured mouse possessing a low blood sugar, 78 mg., from family *C* with a piebald chocolate and white, also having a low blood sugar, 80 mg., from family *D* gave a second generation of 47, constituted of 3 albinos with high blood sugars, 118 to 120 mg., 9 albinos with low blood sugars, 78 to 84 mg., 9 coloured animals with high blood sugars, 116 to 120 mg., and 26 coloured with low blood sugars, 74 to 88 mg.



Family J. The mating of two other mice from the same families, *C* and *D*, one a chocolate having a low blood sugar, 82 mg., and the other a chocolate and brown piebald also with a low blood sugar, 80 mg., resulted in a family of 32. Classified according to colour and the sugar content of the fasting blood, these showed 2 albinos with high blood sugars, 116 to 120 mg., 6 albinos with low blood sugars, 78 to 84 mg., 6 coloured with high blood sugars, 113 to 120 mg., and 18 coloured with low blood sugars, 76 to 84 mg.



Family K. In a third experiment with members of families *C* and *D*, a chocolate coloured mouse possessing a low fasting blood sugar, 80 mg., was crossed with a piebald chocolate and white, also showing a low fasting blood sugar, 82 mg., and a second generation of 30 resulted. Two of these were albinos with high blood sugars, 118 to 120 mg., 6 were albinos with low blood sugars, 78 to 84 mg., 6 were coloured and had high blood sugars, 116 to 120 mg., while 16 were coloured but showed low blood sugars, 78 to 88 mg.



Families *H*, *J*, *K* added together give:

A.H.	A.L.	C.H.	C.L.
7	21	21	60
where 6.8	20.4	20.4	61.2

is the expectation.

The findings obtained with the second generation confirmed the conclusion that hyperglycaemia, like albinism, is a recessive character, for examination of the figures showed that they were in close accord with those required for such a condition by the Mendelian theory. They further demonstrated quite distinctly that, although both hyperglycaemia and albinism are recessive characters, they are genetically independent, as the one is not necessarily associated with the other. It would therefore seem that the factors giving rise to albinism do not include those leading to an abnormally high blood sugar as our original observations suggested might possibly be the case. The numbers moreover dispose of any idea of linkage between the two factors. The association originally noticed must therefore have been an accidental feature of the strains used. It is clearly a matter of considerable interest and importance, however, that a definite chemical abnormality, such as hyperglycaemia, should have been proved experimentally to be transmitted in accordance with Mendel's theory of heredity. Evidence suggesting that certain other abnormalities of metabolism may be transmitted according to Mendel's laws has been collected and published by several observers, but as their investigations have been confined to a study of the family histories of cases presenting such chemical defects the data have necessarily been incomplete.

Bateson¹ and Punnett² have pointed out that the incidence of alcaptonuria in human beings suggests that it is a recessive character in the Mendelian sense, and Garrod³ has collected histories of cases indicating that congenital steatorrhoea, congenital haematoporphyrin, pentosuria and cystinuria may be of a similar type.

We wish to acknowledge our indebtedness to Sir F. W. Andrewes for permission to carry out the experimental part of this work in the Pathological Department of St Bartholomew's Hospital, and also to thank Professor Bateson for very kindly revising the proofs of this paper.

¹ *Report of the Evolution Committee of the Royal Society*, 1902, No. 1, p. 133 note.

² *Proc. of the Royal Soc. of Med.* 1908, 1. Epidem. Sect., p. 148.

³ *Inborn Errors of Metabolism*, Oxford Med. Publications, 1923.